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**(54) Title: DNA ENCODING 5-HT<sub>4</sub> SEROTONIN RECEPTORS AND USES THEREOF**

### (S7) Abstract

This invention provides an isolated nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor and an isolated nucleic acid molecule encoding a human 5-HT<sub>4</sub> receptor, an isolated protein which is a mammalian 5-HT<sub>4</sub> receptor, an isolated protein which is a human 5-HT<sub>4</sub> receptor, vectors comprising an isolated nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor, vectors comprising an isolated nucleic acid molecule encoding a human 5-HT<sub>4</sub> receptor, mammalian cells comprising such vectors, antibodies directed to the 5-HT<sub>4</sub> receptor, nucleic acid probes useful for detecting nucleic acid encoding a mammalian or human 5-HT<sub>4</sub> receptor, antisense oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a mammalian or human 5-HT<sub>4</sub> receptor, pharmaceutical compounds related to the human 5-HT<sub>4</sub> receptor, and nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian or human 5-HT<sub>4</sub> receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatments for alleviating abnormalities associated with a human 5-HT<sub>4</sub> receptor.

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DNA ENCODING 5-HT<sub>1</sub> SEROTONIN RECEPTORS AND USES THEREOF

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Background of the Invention

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Throughout this application various publications are referred to by partial citations within parenthesis. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications, in their entireties, are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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Molecular cloning efforts have provided primary amino acid sequence and signal transduction data for a large collection of serotonin receptor subtypes. These include five cloned 5-HT<sub>1</sub>-like receptors, three cloned 5-HT<sub>2</sub> receptors, and one 5-HT<sub>3</sub> receptor. The 5-HT<sub>1</sub> subfamily includes: 5-HT<sub>1A</sub> (Fargin, 1988; Kobilka, 1989), 5-HT<sub>1B</sub>/5-HT<sub>1D</sub> (Weinshank et al., 1991; Demchyshyn et al., 1992; Jin et al., 1992; Adham et al., 1992; Maroteaux et al., 1992; Voight et al., 1991), 5-HT<sub>1D</sub> (Branchek et al. 1991; Hamblin and Metcalf, 1991; Weinshank et al., 1992), 5-HT<sub>1E</sub> (Levy et al., 1992; McAllister et al., 1992; Zgombick et al., 1992) and 5-HT<sub>1F</sub> (Adham et al., 1993). All five have been shown to couple to the inhibition of adenylate cyclase activity. The 5-HT<sub>2</sub> family includes the 5-HT<sub>2</sub> receptor (Pritchett et al., 1988), 5-HT<sub>1C</sub> (Julius et al., 1989) and 5-HT<sub>2F</sub> (Rat Stomach Fundus; Foquet et al., 1992; Kursar et al., 1992). These receptors all couple to phosphoinositide hydrolysis. The 5-HT<sub>3</sub> receptor is a ligand-gated ion channel (Maricq et al., 1991).

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Although this work represents enormous success, the absence of molecular biological information on the 5-

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HT<sub>7</sub> receptors, which have been shown in native tissues to couple to the activation of adenylate cyclase as a primary mode of signal transduction (Dumuis et al., 1988; Bockaert et al., 1990), is apparent. In a previous copending application (U.S. Serial No., 971,690, filed November 3, 1992), we reported the cloning of the first mammalian 5-HT receptor that couples to the stimulation of adenylate cyclase activity which we named 5-HT<sub>4B</sub>. The 5-HT<sub>4B</sub> receptor was subsequently renamed to the "5-HT<sub>7</sub> receptor" by the "Serotonin Receptor Nomenclature Committee" of the IUPHAR. The pharmacological properties of this receptor indicated that it was similar to a series of functionally defined 5-HT receptors described in the porcine vena cava (Trevethick et al., 1984), cat saphenous vein, coronary arteries (Cushing and Cohen, 1992), and several vascular dilatory effects (Mylecharane and Phillips, 1989). However, the classically defined 5-HT<sub>4</sub> receptor remained to be cloned. We now report the cloning of the pharmacologically-defined 5-HT<sub>4</sub> receptor which we have previously called 5-HT<sub>4A</sub> and now designate as the 5-HT<sub>4</sub> receptor. This receptor also stimulates adenylate cyclase activity but unlike 5-HT<sub>4B</sub>, is sensitive to a series of benzamide derivatives which act as agonists or partial agonists at this subtype. The presence of this subtype in the brain, particularly in areas such as the hippocampus, indicates a potential role in cognitive enhancement. In addition, the 5-HT<sub>4</sub> receptor has been described functionally in the heart, adrenal, bladder, and alimentary canal indicating potential roles in achalasia, hiatal hernia, esophageal spasm, irritable bowel disease, postoperative ileus, diabetic gastroparesis, emesis and other diseases of the gastrointestinal tract, as well as in cardiac, urinary, and endocrine function.

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Summary of the Invention

This invention provides an isolated nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor. In a preferred embodiment of this invention, the isolated nucleic acid encodes a human 5-HT<sub>4</sub> receptor. In another embodiment of this invention, the nucleic acid molecule encoding a human 5-HT<sub>4</sub> receptor comprises a plasmid designated pBluescript-hS10 (ATCC Accession No. 75392). In another embodiment of this invention a nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor comprises a plasmid designated pcEXV-S10-87 (ATCC Accession No. 75390). In another embodiment of this invention a nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor comprises a plasmid designated pcEXV-S10-95 (ATCC Accession No. 75391).

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor. This invention also provides a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>4</sub> receptor.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a mammalian 5-HT<sub>4</sub> receptor so as to prevent translation of the mRNA molecule. This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a human 5-HT<sub>4</sub> receptor so as to prevent translation of the mRNA molecule.

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This invention provides a monoclonal antibody directed to a mammalian 5-HT<sub>4</sub> receptor. This invention also provides a monoclonal antibody directed to a human 5-HT<sub>4</sub> receptor.

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This invention provides a pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a mammalian 5-HT<sub>4</sub> receptor and a pharmaceutically acceptable carrier. This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of mammalian 5-HT<sub>4</sub> receptor and a pharmaceutically acceptable carrier.

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This invention provides a pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human 5-HT<sub>4</sub> receptor and a pharmaceutically acceptable carrier. This invention also provides pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a human 5-HT<sub>4</sub> receptor and a pharmaceutically acceptable carrier.

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This invention provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian 5-HT<sub>4</sub> receptor so positioned within such genome as to be transcribed into antisense mRNA complementary to mRNA encoding the mammalian 5-HT<sub>4</sub> receptor and when hybridized to mRNA encoding the mammalian 5-HT<sub>4</sub> receptor, the complementary mRNA reduces the translation of the mRNA encoding the mammalian 5-HT<sub>4</sub> receptor.

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This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human 5-HT<sub>4</sub> so positioned within such genome as to be transcribed into antisense mRNA complementary to mRNA encoding the human 5-HT<sub>4</sub>, and when hybridized to mRNA encoding the human 5-HT<sub>4</sub>, the complementary mRNA reduces the translation of the mRNA encoding the human 5-HT<sub>4</sub>.

10 This invention provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian 5-HT<sub>4</sub> receptor so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the mammalian 5-HT<sub>4</sub> receptor and when hybridized to mRNA encoding the 5-HT<sub>4</sub> receptor, the antisense mRNA thereby prevents the translation of mRNA encoding the 5-HT<sub>4</sub> receptor.

20 This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human 5-HT<sub>4</sub> receptor so positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the human 5-HT<sub>4</sub> receptor and when hybridized to mRNA encoding the human 5-HT<sub>4</sub> receptor, the antisense mRNA thereby prevents the translation of mRNA encoding the human 5-HT<sub>4</sub> receptor.

25 This invention also provides a method of determining the physiological effects of expressing varying levels of a mammalian 5-HT<sub>4</sub> receptor which comprises producing a transgenic nonhuman animal whose levels of mammalian 5-HT<sub>4</sub> receptor expression are varied by use of an inducible promoter which regulates mammalian 5-HT<sub>4</sub> receptor expression.

35 This invention also provides a method of determining the physiological effects of expressing varying lev ls

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of a human 5-HT<sub>4</sub> receptor which comprises producing a transgenic nonhuman animal whose levels of human 5-HT<sub>4</sub> receptor expression are varied by use of an inducible promoter which regulates human 5-HT<sub>4</sub> receptor expression.

This invention further provides a method of determining the physiological effects of expressing varying levels of mammalian 5-HT<sub>4</sub> receptor which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of mammalian 5-HT<sub>4</sub> receptor.

This invention further provides a method of determining the physiological effects of expressing varying levels of human 5-HT<sub>4</sub> receptor which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human 5-HT<sub>4</sub> receptor.

This invention provides a method for determining whether a compound not known to be capable of specifically binding to a human 5-HT<sub>4</sub> receptor can specifically bind to the human 5-HT<sub>4</sub> receptor, which comprises contacting a mammalian cell comprising a plasmid adapted for expression in a mammalian cell which plasmid further comprises a DNA which expresses a human 5-HT<sub>4</sub> receptor on the cell's surface with the compound under conditions permitting binding of ligands known to bind to a human 5-HT<sub>4</sub> receptor, detecting the presence of any compound bound to the human 5-HT<sub>4</sub> receptor, the presence of bound compound indicating that the compound is capable of specifically binding to the human 5-HT<sub>4</sub> receptor.

This invention provides a method of screening drugs to identify drugs which interact with, and specifically bind to, a human 5-HT<sub>4</sub> receptor on the surface of a cell, which comprises contacting a mammalian cell which

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comprises a plasmid adapted for expression in a mammalian cell which plasmid further comprises DNA which expresses a human 5-HT<sub>4</sub> receptor on the cell's surface with a plurality of drugs, determining those drugs which bind to the human 5-HT<sub>4</sub> receptor expressed on the cell surface of the mammalian cell, and thereby identifying drugs which interact with, and specifically bind to, the human 5-HT<sub>4</sub> receptor.

This invention provides a method for identifying a compound which specifically binds to and activates or blocks the activation of a human 5-HT<sub>4</sub> receptor on the surface of a mammalian cell, which comprises contacting the mammalian cell which comprises a plasmid adapted for expression in the mammalian cell such plasmid further comprising DNA which expresses the human 5-HT<sub>4</sub> receptor on the cell surface of the mammalian cell with the compound, determining whether the compound activates or blocks the activation of the human 5-HT<sub>4</sub> receptor and thereby identifying the compound as a compound which binds to, and activates or blocks the activation of the human 5-HT<sub>4</sub> receptor.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a human 5-HT<sub>4</sub> receptor allele which comprises: a.) obtaining DNA of subjects suffering from the disorder; b.) performing a restriction digest of the DNA with a panel of restriction enzymes; c.) electrophoretically separating the resulting DNA fragments on a sizing gel; d.) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a 5-HT<sub>4</sub> receptor and labelled with a detectable marker; e.) detecting labelled bands which have hybridized to the DNA encoding a 5-HT<sub>4</sub> receptor labelled with a detectable marker to create a unique band pattern specific to the

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DNA of subjects suffering from the disorder; f.) preparing DNA obtained for diagnosis by steps a-e; and g.) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

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Brief Description f Figur s

5      **Figure 1:** Nucleotide and corresponding amino acid sequence of the S10-87 cDNA clone. Only partial 5' and 3' untranslated sequences are shown.

10     **Figure 2:** Nucleotide and corresponding amino acid sequence of the S10-95 cDNA clone. Only partial 5' and 3' untranslated sequences are shown.

15     **Figure 3:** Comparison of amino acid sequences between clones S10-87 (top row) and S10-95 (bottom row). The overall homology is 96.7%.

20     **Figure 4:** Comparison of the rat S10 receptor deduced amino acid sequences with those of other serotonin receptors and with the canine histamine H<sub>2</sub> receptor. Solid bars, the seven putative membrane-spanning domains (TM I-VII). Shading, homologies between the S10 receptors and other receptors. Hp78, 5-HT4B or hp78a receptor (U.S. Serial No., 971,960, filed, November 3, 1992, copending).

25     **Figure 5:** Nucleotide and amino acid sequences of the human S10 PCR clone. The numbering is given according to the rat S10-95 clone.

30     **Figure 6:** Comparison of nucleotide sequences between the human PCR S10 clone and the rat S10 cDNA clone. Top row: human sequence, the numbering is given according to the rat S10 nucleotide sequence. The bottom row outlines differences in the rat sequence (overall homology: 90.7%).

35     **Figure 7:** Comparison of deduced amino acid sequences between the Human S10 PCR clone and the rat S10 cDNA clone. Top row: human S10 sequence, the numbering is

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given according to the rat S10 amino acid sequence. The bott m r w outlines differences in the rat sequence (overall homology: 92.3 %).

5      **Figure 8:** Comparison of binding affinities of key compounds at the S10 clone with adenylate cyclase functional responses obtained with mouse collicular neurons. A correlation plot was constructed between affinity constants of drugs for the S10 receptor with those obtained at a pharmacologically defined 5-HT<sub>4</sub> receptor. Binding values for the correlation were taken from table 1 and were expressed as the negative logarithm. Functional data were taken from Dumuis et al. (1988). The correlation coefficient calculated by linear regression was 0.96 indicating that the rank order of potency for the compounds was similar in both preparations.

20     **Figure 9:** Stimulation of cAMP production by 5-HT in transiently transfected Cos-7 cells expressing the cloned rat 5-HT<sub>4</sub> (CG-7) receptor and antagonism by ICS 205930. cAMP measurements on intact cells were as described under Methods and Materials. Each data point represents the mean of triplicates from a single experiment representative of at least 2 others. The vertical bars indicate S.E.M. Data are presented as percent maximum cAMP released by 5-HT (basal cAMP release: 0.020 ± 0.002 pmol/ml/10 min; maximum cAMP release: 0.42 ± 0.03 pmol/ml/10 min).

30     **Figure 10:** Stimulation of cAMP production by 5-HT in transiently transfected Cos-7 cells expressing the cloned rat 5-HT<sub>4</sub> (CG-8) receptor and antagonism by ICS 205930. cAMP measurements on intact cells were as described under Methods and Materials. Each data point represents the mean of triplicates from a single experiment representative of at least two others. The

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vertical bars indicate S.E.M. Data are presented as percent maximum cAMP released by 5-HT (basal cAMP release:  $0.023 \pm 0.004$  pmol/ml/10 min; maximum cAMP release:  $0.57 \pm 0.04$  pmol/ml/10 min).

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**Figure 11A:** Nucleotide sequence of the partial human S10-87 clone. Only partial 3' untranslated sequences are shown (SEQ. ID NO. 14).

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**Figure 11B:** Deduced amino acid sequence encoded by the nucleotide sequence of Figure 11A of the partial human S10-87 clone (SEQ. ID NO. 15).

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**Figure 12:** Comparison of the nucleotide sequences between the human (top row) and the rat S10-87 (bottom row) cDNA clones. The overall identity is 90.8%.

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**Figure 13:** Comparison of the deduced amino acid sequences between the human (top row) and the rat (bottom row) S10-87 receptors. The overall identity is 93.9%.

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**Figure 14A:** Nucleotide sequence of the full length human S10-95 clone (SEQ. ID NO. 7).

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**Figure 14B:** Deduced amino acid sequence encoded by the nucleotide sequence of Figure 14A (SEQ. ID NO. 8).

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**Figure 15:** Comparison of the nucleotide sequences between the human (top row) and the rat (bottom row) S10-95 cDNA clones. The overall identity is 90.7%.

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**Figure 16:** Comparison of the deduced amino acid sequences between the human (top row) and the rat (bottom row) S10-95 receptors. The overall identity is 93.8%.

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Figure 17: Comparison of the nucleotide sequences corresponding to the available coding regions between the two human isoforms (top row S10-95; bottom row S10-87) of the 5-HT<sub>2</sub> receptor. The overall identity is 92%.

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Figure 18: Comparison of the deduced amino acid sequences between the two human isoforms (top row S10-95; bottom row S10-87) of the 5-HT<sub>2</sub> receptor. The overall identity is 90%.

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Figure 19: Inhibition of [<sup>3</sup>H]GR11380 binding on the cloned rat CG-8 receptor by 5-HT<sub>2</sub>, in the absence and presence of Gpp(NH)p (100 μM). Membranes harvested from transient transfectants (COS-7 cells) were incubated with [<sup>3</sup>H]IGR113808 (0.2-0.4 nM) for 30 min at 37°C. Nonspecific binding was defined by 50 μM unlabelled 5-HT. Data are from a single experiment. Data were analyzed by computer-assisted nonlinear regression analysis (Accufit; Landon Software).

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Detail d Description of the Invention

This invention provides an isolated nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor. This invention further provides an isolated nucleic acid molecule encoding a human 5-HT<sub>4</sub> receptor. As used herein, the term "isolated nucleic acid molecule" means a non-naturally occurring nucleic acid molecule that is, a molecule in a form which does not occur in nature. Examples of such an isolated nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a mammalian 5-HT<sub>4</sub> receptor or a human 5-HT<sub>4</sub> receptor. As used herein, "5-HT<sub>4</sub> receptor" means a molecule which, under physiologic conditions, is substantially specific for the neurotransmitter serotonin, is saturable, of high affinity for serotonin and the activation of which is coupled to the activation of adenylate cyclase and the "5-HT<sub>4</sub> receptor" is also sensitive to benzamide derivatives which act as agonists and partial agonists at this receptor subtype. One embodiment of this invention is an isolated nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor. Such a molecule may have coding sequences substantially the same as the coding sequences shown in Figures 1 and 2 and 5 (SEQ ID NOS. 1, 3 and 5). A preferred embodiment is an isolated nucleic acid molecule encoding a human 5-HT<sub>4</sub> receptor. Such a molecule may have a coding sequence substantially the same as the coding sequence shown in Figure 5 (SEQ ID NO. 5). The DNA molecules of Figures 1, 2 and 5 (Seq ID NOS. 1, 3 and 5) encode the sequence of mammalian 5-HT<sub>4</sub> receptors. The DNA molecule of Figure 5 (Seq ID No. 5) encodes a human 5-HT<sub>4</sub> receptor. This invention further provides isolated DNA molecules encoding mammalian 5-HT<sub>4</sub> receptors having the sequence H<sub>2</sub>N-Y-X-COOH wherein Y is the amino acid sequence beginning at amino acid 1 and ending at amin

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acid 359 of Figure 1 (SEQ ID NOS. 1 and 2) and wherein X is an amino acid sequence encoding the carboxy terminal region of the receptor. The nucleic acid molecules of Figures 1 and 2 (SEQ ID NOS 1-4) encode 5-HT<sub>4</sub> receptors having an identical sequence Y and differing only in their carboxy terminal region X beginning at amino acid 360. One means of isolating a nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor is to probe a mammalian genomic library with a natural or artificially designed DNA probe, using methods well known in the art. In the preferred embodiment of this invention, the mammalian 5-HT<sub>4</sub> receptor is a human protein and the nucleic acid molecule encoding the human 5-HT<sub>4</sub> receptor is isolated from human cDNA. Degenerate oligonucleotide primers derived from transmembrane (TM) domains of 5-HT<sub>1A</sub>, 5-HT<sub>1C</sub>, 5-HT<sub>2</sub> and 5-HT<sub>1B/5</sub> receptors are useful for identifying cDNA containing a nucleic acid molecule encoding a 5-HT<sub>4</sub> receptor, obtaining a probe specific to a mammalian 5-HT<sub>4</sub> receptor and for isolating a nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor.

DNA and cDNA molecules which encode a mammalian 5-HT<sub>4</sub> receptor are used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5' untranslated region of the isolated clone, and other stability, processing, transcription, translation, and tissue specificity determining regions from the 3' and 5' untranslated regions of the isolated gene are thereby obtained.

This invention provides an isolated nucleic acid

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molecule which has a nucleic acid sequence which differs from the sequence of a nucleic acid molecule encoding a 5-HT<sub>4</sub> receptor at one or more nucleotides and which does not encode a protein having 5-HT<sub>4</sub> receptor activity. As used herein, "5-HT<sub>4</sub> receptor activity" means the capability of receptor to specifically bind the neurotransmitter, serotonin under physiological conditions and the capability of the receptor to activate adenylyl cyclase when the receptor is coupled to adenylyl cyclase. An example of a isolated nucleic acid molecule provided by this invention is a nucleic acid molecule which has an in-frame stop codon inserted into the coding sequence such that the transcribed RNA is not translated into protein.

This invention further provides a cDNA molecule encoding a mammalian 5-HT<sub>4</sub> receptor, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figures 1, 2 and 5 (Seq ID NOS. 1, 3 and 5). This invention provides a cDNA molecule encoding a human 5-HT<sub>4</sub> receptor, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figure 5 (SEQ ID NO. 5). These molecules and their equivalents were obtained by the means described above.

This invention also provides an isolated protein which is a mammalian 5-HT<sub>4</sub> receptor. In a preferred embodiment of this invention, the protein is a human 5-HT<sub>4</sub> receptor protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figures 1, 2 and 5 (SEQ ID Nos. 1-6). In another embodiment of this invention, the protein is a murine 5-HT<sub>4</sub> receptor protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figures 1, 2 and 5 (SEQ ID NOS. 1-6). As used

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herein, the term "isolated protein" is intended to encompass a protein molecule free of other cellular components. One means for obtaining an isolated mammalian 5-HT<sub>2</sub> receptor protein is to express DNA encoding the 5-HT<sub>2</sub> receptor in a suitable host, such as a bacterial, yeast, insect, or mammalian cell, using methods well known to those skilled in the art, and recovering the receptor protein after it has been expressed in such a host, again using methods well known in the art. The receptor may also be isolated from cells which express it, in particular from cells which have been transfected with the expression vectors described below in more detail.

This invention provides a vector comprising DNA, RNA, or cDNA, encoding a mammalian 5-HT<sub>2</sub> receptor. This invention further provides a vector comprising DNA, RNA, or cDNA, encoding a human 5-HT<sub>2</sub> receptor. Examples of vectors are viruses such as bacteriophages (such as phage lambda), cosmids, plasmids (such as pUC18, available from Pharmacia, Piscataway, NJ), and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known to those skilled in the art. Examples of such plasmids are plasmids comprising DNA having a coding sequence substantially the same as the coding sequence shown in Figures 1, 2 and 5 (SEQ ID NOS. 1, 3 and 5) and designated pcEXV-S10-87 (ATCC Accession No. 75390), pcEXV-S10-95 (ATCC Accession No. 75391) and pBLUEScript-hS10 (ATCC No. 75392).

Alternatively, to obtain these vectors, insert and vector DNA can both be exposed to a restriction enzyme to create complementary ends on both molecules which base pair with each other and are then ligated together with a ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction

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site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other enzymes are also available.

5 This invention also provides vectors comprising a DNA or cDNA encoding a mammalian 5-HT<sub>4</sub> receptor and vectors comprising a DNA or cDNA encoding a human 5-HT<sub>4</sub> receptor, adapted for expression in a bacterial cell, a yeast cell, insect cell or a mammalian cell which 10 additionally comprise the regulatory elements necessary for expression of the DNA or cDNA encoding a mammalian 5-HT<sub>4</sub> receptor or the DNA or cDNA encoding a human 5-HT<sub>4</sub> receptor in the bacterial, yeast, insect or mammalian cells operatively linked to the DNA or cDNA encoding 15 the 5-HT<sub>4</sub> receptor as to permit expression thereof. DNA or cDNA having coding sequence substantially the same as the coding sequence shown in Figures 1 and 2 (SEQ ID NOS. 1 and 3) may be usefully inserted into these vectors to express a mammalian 5-HT<sub>4</sub> receptor. 20 DNA or cDNA having a coding sequence substantially the same as the coding sequence shown in Figure 5 (SEQ ID NO. 5) may be usefully inserted into these vectors to express the human 5-HT<sub>4</sub> receptor. Regulatory elements required for expression include promoter sequences to 25 bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Maniatis, et al., 1982). Similarly, a eukaryotic 30 expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Furthermore, an insect expression vector, such as 35 recombinant Baculovirus, uses the polyhedrin gene expression signals for expression of the inserted gene

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in insect cells. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express receptors. Certain uses for such cells are described in more detail below.

In one embodiment of this invention a plasmid is adapted for expression in a bacterial, yeast, insect, or, in particular, a mammalian cell wherein the plasmid comprises DNA or cDNA encoding a mammalian 5-HT<sub>4</sub> receptor or DNA or cDNA encoding a human 5-HT<sub>4</sub> receptor and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, insect, or mammalian cell operatively linked to the DNA or cDNA encoding a mammalian 5-HT<sub>4</sub> receptor or to the DNA or cDNA encoding a human 5-HT<sub>4</sub> receptor as to permit expression thereof. Suitable plasmids may include, but are not limited to plasmids adapted for expression in a mammalian cell, e.g., EVJB, EXV-3. An example of such a plasmid adapted for expression in a mammalian cell is a plasmid comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figures 1, 2 and 5 (SEQ ID NOS. 1, 3 and 5) and the regulatory elements necessary for expression of the DNA in the mammalian cell. These plasmids have been designated pcEXV-S10-87 deposited under ATCC Accession No. 75390, pcEXV-S10-95 deposited under ATCC Accession No. 75391, and pBluescript-hS10, deposited under ATCC Accession No. 75392. Those skilled in the art will readily appreciate that numerous plasmids adapted for expression in a mammalian cell which comprise DNA encoding a mammalian or human 5-HT<sub>4</sub> receptor and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing plasmids and adapted as appropriate to contain

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th regulatory elements necessary to express the DNA in  
the mammalian cell. The plasmids may be constructed by  
th meth ds described above for expression vectors and  
vectors in general, and by other methods well known in  
5 the art.

Deposit discussed supra were made pursuant to, and in  
satisfaction of, the provisions of the Budapest Treaty  
on the International Recognition of the Deposit of  
10 Microorganisms for the Purpose of Patent Procedure with  
the American Type Culture Collection (ATCC), 12301  
Parklawn Drive, Rockville, Maryland 20852.

This invention provides a mammalian cell comprising a  
15 DNA or cDNA molecule encoding a mammalian 5-HT<sub>4</sub>  
receptor, such as a mammalian cell comprising a plasmid  
adapted for expression in a mammalian cell, said  
plasmid further comprises DNA or cDNA encoding a  
mammalian 5-HT<sub>4</sub> receptor and the regulatory elements  
20 necessary for expression of the DNA or cDNA in the  
mammalian cell operatively linked to the DNA or cDNA  
encoding a mammalian 5-HT<sub>4</sub> receptor as to permit  
expression thereof. This invention provides a mammalian  
cell comprising a DNA or cDNA molecule encoding a human  
25 5-HT<sub>4</sub> receptor, such as a mammalian cell comprising a  
plasmid adapted for expression in a mammalian cell,  
said plasmid further comprises a DNA or cDNA molecule  
encoding a human 5-HT<sub>4</sub> receptor and the regulatory  
elements necessary for expression of the DNA or cDNA in  
30 the mammalian cell operatively linked to the DNA or  
cDNA encoding a human 5-HT<sub>4</sub> receptor as to permit  
expression thereof. Numerous mammalian cells may be  
used as hosts, including, but not limited to, the mouse  
fibroblast cell NIH3T3, CHO cells, HeLa cells, LM (tk-)  
35 cells, Cos-7 cells, etc. Expression plasmids such as  
that described supra may be used to transfect mammalian  
cells by methods well known in the art such as calcium

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phosphate precipitation, or DNA or cDNA encoding a human or mammalian 5-HT<sub>4</sub> receptor may be otherwise introduced into mammalian cells, e.g., by microinjection, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding a human or mammalian 5-HT<sub>4</sub> receptor.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with an unique sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>4</sub> receptor, for example with a coding sequence included within the sequences shown in Figure 5 (SEQ ID NO. 5). This invention further provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor, for example with a coding sequence included within the sequences shown in Figure 1 and Figure 2 (SEQ ID NOS. 1 and 3) As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. As used herein, the phrase "unique sequence" means a nucleic acid molecule sequence specific to only the nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid encoding a human 5-HT<sub>4</sub> receptor is useful as a diagnostic test for any disease process in which levels of expression of the 5-

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HT<sub>4</sub> receptor are altered. DNA probe molecules are produced by insertion of a DNA molecule which encodes a 5-HT<sub>4</sub> receptor or fragments thereof into suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the DNA probes, all using methods well known in the art. For example, the DNA may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the DNA into the vector (discussed above), electrophoresed, and cut out of the resulting gel. An example of such DNA molecules is shown in Figures 1, 2 and 5 (SEQ ID NOS. 1, 3, and 5). The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA molecule which encode a mammalian 5-HT<sub>4</sub> receptor or complementary to the sequence of a DNA molecule which encodes a human 5-HT<sub>4</sub> receptor are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by the use of amplification techniques such as the polymerase chain reaction.

This invention also provides a method of detecting expression of a human 5-HT<sub>4</sub> receptor on the surface of a cell by detecting the presence of mRNA coding for a 5-HT<sub>4</sub> receptor. This invention further provides a method of detecting expression of a mammalian 5-HT<sub>4</sub> receptor on the surface of the cell by detecting the presence of mRNA coding for a mammalian 5-HT<sub>4</sub> receptor. These methods comprise obtaining total mRNA from the cell using methods well known in the art and contacting

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the mRNA so obtained with a nucleic acid probe as described hereinabove, under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the receptor by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well known in the art. However, in one embodiment of this invention, nucleic acids are extracted by precipitation from lysed cells and the mRNA is isolated from the extract using a column which binds the poly-A tails of the mRNA molecules (Maniatis et al., 1982). The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding may be detected by autoradiography or scintillation counting. However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human 5-HT<sub>4</sub> receptor so as to prevent translation of the human 5-HT<sub>4</sub> receptor. The antisense oligonucleotide may have a sequence capable of binding specifically with any sequences of the cDNA molecule whose sequence is shown in Figure 5 (SEQ ID NO. 5). This invention also provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a mammalian 5-HT<sub>4</sub> receptor so as to prevent translation of the mammalian 5-HT<sub>4</sub> receptor. The antisense oligonucleotide may have a sequence capable of binding specifically with any sequences of the cDNA molecule whose sequence is shown in Figures 1 and 2 (SEQ ID NOS. 1 and 3). As used herein, the phrase "binding specifically" means the

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ability of an antisense oligonucleotide to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. A particular example 5 of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogues of nucleotides.

This invention also provides a pharmaceutical 10 composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a human 5-HT<sub>4</sub> receptor by passing through a cell membrane and binding specifically with mRNA encoding the 5-HT<sub>4</sub> receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing 15 through a cell membrane. This invention further provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above 20 effective to reduce expression of a mammalian 5-HT<sub>4</sub> receptor by passing through a cell membrane and binding specifically with mRNA encoding a mammalian 5-HT<sub>4</sub> receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier 25 capable of passing through a cell membrane. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil 30 emulsion, and various types of wetting agents. The oligonucleotide may be coupled to a substance which inactivates mRNA, such as a ribozyme. The pharmaceutically acceptable hydrophobic carrier capable 35 of passing through cell membranes may also comprise a structure which binds to a transporter specific for a selected cell type and is thereby taken up by cells of the selected cell type. The structure may be part of

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a protein known to bind a cell-type specific transporter, for example an insulin molecule, which would target pancreatic cells. DNA molecules having a coding sequence substantially the same as the coding sequences shown in Figure 5 (SEQ ID No. 5) may be used as the oligonucleotides of the pharmaceutical composition.

This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a mammalian 5-HT<sub>4</sub> receptor by passing through a cell membrane and binding specifically with mRNA encoding the 5-HT<sub>4</sub> receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. DNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1 and 2 (SEQ ID NOS. 1 and 3) may be used as the oligonucleotides of the pharmaceutical composition.

This invention provides a method of treating abnormalities which are alleviated by reduction of expression of 5-HT<sub>4</sub> receptor. This method comprises administering to a subject an effective amount of the pharmaceutical composition described above effective to reduce expression of the 5-HT<sub>4</sub> receptor by the subject. This invention further provides a method of treating an abnormal condition related to 5-HT<sub>4</sub> receptor activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to reduce expression of the 5-HT<sub>4</sub> receptor by the subject. Examples of such abnormal conditions are irritable bowel disease, postoperative ileus, diabetic gastroparesis, emesis, achalasia, hiatal hernia, esophageal spasm and other diseases of the

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gastrointestinal tract, as well as in cardiac, urinary, and endocrine function.

5       Antisense oligonucleotide drugs inhibit translation of mRNA encoding 5-HT<sub>4</sub> receptor. Synthetic antisense oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding the 5-HT<sub>4</sub> receptor and inhibit translation of mRNA and are useful as drugs to inhibit expression of 5-HT<sub>4</sub> receptor genes in patients. This invention provides a means to therapeutically alter levels of expression of a human or mammalian 5-HT<sub>4</sub> receptor by the use of a synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding the 5-HT<sub>4</sub> receptor.

10      15     Synthetic antisense oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the nucleotide sequence shown in Figure 1, 2 and 5 (SEQ ID NOS. 1, 3 and 5) of DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory cell culture conditions, for administration to cells removed from the patient. The

20      25     SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable of passing through cell membranes (e.g., by designing small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain selected cell populations by targeting the SAOD to be recognized by specific cellular uptake mechanisms which bind and take up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to

30      35

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bind to transporter found only in a certain cell type, as discussed above. The SAOD is also designed to recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequence shown in Figure 1, 2 and 5 (SEQ ID NOS. 1, 3, and 5) by virtue of complementary base pairing to the mRNA. Finally, the SAOD is designed to inactivate the target mRNA sequence by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular mechanisms such as RNase I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA targets (Cohen, J.S., 1989; Weintraub, H.M., 1990). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target mRNA (N. Sarver et al., 1990). An SAOD serves as an effective therapeutic agent if it is designed to be administered to a patient by injection, or if the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce 5-HT<sub>4</sub> receptor expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of 5-HT<sub>4</sub> receptor.

This invention provides an antibody directed to the human 5-HT<sub>4</sub> receptor. This invention also provides an antibody directed to the mammalian 5-HT<sub>4</sub> receptor. This antibody may comprise, for example, a monoclonal

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antibody directed to an epitope of a human 5-HT<sub>4</sub> receptor present on the surface of a cell, the epitope having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human 5-HT<sub>4</sub> receptor included in the amino acid sequence shown in Figure 5. Amino acid sequences may be analyzed by methods well known to those skilled in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to form the part of the protein that is inserted into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figure 5 will bind to a surface epitope of a 5-HT<sub>4</sub> receptor as described. Antibodies directed to a human or mammalian 5-HT<sub>4</sub> receptor may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as NIH3T3 cells or LM (tk<sup>-</sup>) cells may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequence shown in Figures 1, 2, and 5 (SEQ ID NOS. 1-6). As a still further alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of 5-HT<sub>4</sub> receptor encoded by the isolated DNA, or to inhibit the function of the 5-HT<sub>4</sub> receptor in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

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This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of the human 5-HT<sub>4</sub> receptor, effective to block binding of naturally occurring substrates to the 5-HT<sub>4</sub> receptor, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a human 5-HT<sub>4</sub> receptor present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human 5-HT<sub>4</sub> receptor included in the amino acid sequence shown in Figure 5 (SEQ ID NOS. 5 and 6) is useful for this purpose.

This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of a mammalian 5-HT<sub>4</sub> receptor, effective to block binding of naturally occurring substrates to the 5-HT<sub>4</sub> receptor, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a mammalian 5-HT<sub>4</sub> receptor present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of a mammalian 5-HT<sub>4</sub> receptor included in the amino acid sequence shown in Figures 1 and 2 (SEQ ID NOS. 1-4) is useful for this purpose.

This invention also provides a method of treating abnormalities in a subject which are alleviated by reduction of expression of a human or mammalian 5-HT<sub>4</sub> receptor which comprises administering to the subject an effective amount of the pharmaceutical composition described above effective to block binding of naturally occurring substrates to the receptor and thereby alleviate abnormalities resulting from overexpression of a human or mammalian 5-HT<sub>4</sub> receptor. Binding of the antibody to the receptor prevents the receptor from

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functioning, thereby neutralizing the effects of over expression. The monoclonal antibodies described above are useful for this purpose. This invention additionally provides a method of treating an abnormal condition related to an excess of 5-HT<sub>4</sub> receptor activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring substrates to the 5-HT<sub>4</sub> receptor and thereby alleviate the abnormal condition. Some examples of abnormal conditions associated with excess 5-HT<sub>4</sub> receptor activity are irritable bowel disease, postoperative ileus, diabetic gastroparesis, emesis, achalasia, hiatal hernia, esophageal spasm and other diseases of the gastrointestinal tract, as well as in cardiac, urinary, and endocrine function.

This invention provides methods of detecting the presence of a 5-HT<sub>4</sub> receptor on the surface of a cell which comprises contacting the cell with an antibody directed to the 5-HT<sub>4</sub> receptor, under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby the presence of the 5-HT<sub>4</sub> receptor on the surface of the cell. Such methods are useful for determining whether a given cell is defective in expression of 5-HT<sub>4</sub> receptors. Bound antibodies are detected by methods well known in the art, for example by binding fluorescent markers to the antibodies and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a human 5-HT<sub>4</sub> receptor and a transgenic nonhuman mammal expressing DNA encoding a

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mammalian 5-HT<sub>4</sub> receptor. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a human or mammalian 5-HT<sub>4</sub> receptor so mutated as to be incapable of normal receptor activity, and not expressing native 5-HT<sub>4</sub> receptor. This invention further provides a transgenic nonhuman mammal whose genome comprises DNA encoding a human 5-HT<sub>4</sub> receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a human 5-HT<sub>4</sub> receptor and which hybridizes to mRNA encoding a 5-HT<sub>4</sub> receptor thereby reducing its translation and a transgenic nonhuman mammal whose genome comprises DNA encoding a mammalian 5-HT<sub>4</sub> receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a mammalian 5-HT<sub>4</sub> receptor and which hybridizes to mRNA encoding a mammalian 5-HT<sub>4</sub> receptor thereby reducing its translation. The DNA may additionally comprise an inducible promoter or additionally comprise tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell types. Examples of DNA are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figure 1, 2 and 5 (SEQ ID NOS. 1, 3, and 5). An example of a transgenic animal is a transgenic mouse. Examples of tissue specificity-determining regions are the metallothionein promotor (Low et al., 1986) and the L7 promotor (Oberdick et al., 1990).

30 Animal model systems which elucidate the physiological and behavioral roles of mammalian receptors are produced by creating transgenic animals in which the expression of a receptor is either increased or decreased, or the amino acid sequence of the expressed receptor protein is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant

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versions of DNA encoding a human 5-HT<sub>1</sub> receptor or homologous animal versions of this gene, by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal (Hogan et al., 1986) or, 2) Homologous recombination (Capecchi M.R., 1989; Zimmer A, and Gruss, P., 1989) of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of the receptor. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native receptor but does express, for example, an inserted mutant receptor, which has replaced the native receptor in the animal's genome by recombination, resulting in underexpression of the receptor. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added receptors, resulting in overexpression of the receptor.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium (Hogan, B. et al. 1986). DNA or cDNA encoding a receptor is purified from a vector (such as plasmids pcEXV-S10-87, pcEXV-S10-95 and pBluescript-hS10 described above) by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of

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the trans-gene. The DNA, in an appropriately buffered solution, is put int a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

Since the normal action of receptor-specific drugs is to activate or to inhibit the receptor, the transgenic animal model systems described above are useful for testing the biological activity of drugs directed against the receptors even before such drugs become available. These animal model systems are useful for predicting or evaluating possible therapeutic applications of drugs which activate or inhibit receptors by inducing or inhibiting expression of the native or trans-gene and thus increasing or decreasing expression of normal or mutant receptors in the living animal. Thus, a model system is produced in which the biological activity of drugs directed against the receptors are evaluated before such drugs become available. The transgenic animals which over or under produce the receptor indicate by their physiological state whether over or under production of the receptor is therapeutically useful.

It is therefore useful to evaluate drug action based on the transgenic model system. One use is based on the fact that it is well known in the art that a drug such as an antidepressant acts by blocking neurotransmitter

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uptake, and ther by increases the amount of  
neur transmitter in the synaptic cleft. The  
physiological result f this action is to stimulat the  
production of less receptor by the affected cells,  
leading eventually to underexpression. Therefore, an  
animal which underexpresses receptor is useful as a  
test system to investigate whether the actions of such  
drugs which result in under expression are in fact  
therapeutic. Another use is that if overexpression is  
found to lead to abnormalities, then a drug which down-  
regulates or acts as an antagonist to the receptor is  
indicated as worth developing, and if a promising  
therapeutic application is uncovered by these animal  
model systems, activation or inhibition of the 5-HT<sub>4</sub>  
receptor is achieved therapeutically either by  
producing agonist or antagonist drugs directed against  
the 5-HT<sub>4</sub> receptor or by any method which increases or  
decreases the expression of this receptor in man.

Further provided by this invention is a method of  
determining the physiological effects of expressing  
varying levels of human or mammalian 5-HT<sub>4</sub> receptors  
which comprises producing a transgenic nonhuman animal  
whose levels of human or mammalian 5-HT<sub>4</sub> receptor  
expression are varied by use of an inducible promoter  
which regulates receptor expression. This invention  
also provides a method of determining the physiological  
effects of expressing varying levels of human or  
mammalian 5-HT<sub>4</sub> receptor which comprises producing a  
panel of transgenic nonhuman animals each expressing a  
different amount of human or mammalian 5-HT<sub>4</sub> receptor.  
Such animals may be produced by introducing different  
amounts of DNA encoding a human or mammalian 5-HT<sub>4</sub>  
receptor into the oocytes from which the transgenic  
animals are developed.

This invention also provides a method for identifying

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a substance capable of alleviating abnormalities resulting from overexpression of a human or mammalian 5-HT<sub>4</sub> receptor comprising administering the substance to a transgenic nonhuman mammal expressing at least one artificially introduced DNA molecule encoding a human or mammalian 5-HT<sub>4</sub> receptor and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a human or mammalian 5-HT<sub>4</sub> receptor. As used herein, the term "substance" means a compound or composition which may be natural, synthetic, or a product derived from screening. Examples of DNA molecules are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequences shown in Figures 1, 2, and 5 (SEQ ID NOS. 1, 3, and 5).

This invention provides a pharmaceutical composition comprising an amount of the substance described supra effective to alleviate the abnormalities resulting from overexpression of 5-HT<sub>4</sub> receptor and a pharmaceutically acceptable carrier.

This invention further provides a method for treating the abnormalities resulting from overexpression of a human or mammalian 5-HT<sub>4</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from overexpression of a human or mammalian 5-HT<sub>4</sub> receptor.

This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human or mammalian 5-HT<sub>4</sub> receptor comprising administering the substance to the transgenic nonhuman mammal described above which expresses only nonfunctional human or mammalian 5-HT<sub>4</sub>

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receptor and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a human or mammalian 5-HT<sub>4</sub> receptor.

5 This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from  
10 underexpression of a human or mammalian 5-HT<sub>4</sub> receptor and a pharmaceutically acceptable carrier.

This invention further provides a method for treating  
15 the abnormalities resulting from underexpression of a human or mammalian 5-HT<sub>4</sub> receptor which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a human or mammalian 5-HT<sub>4</sub> receptor.

20 This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a human or mammalian 5-HT<sub>4</sub> receptor allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human or mammalian 5-HT<sub>4</sub> receptor and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a human or mammalian 5-HT<sub>4</sub> receptor labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and

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g) comparing the unique band pattern specific to the DNA of subj cts suffering from the disorder from step e and th DNA obtained for diagnosis from st p f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human 5-HT<sub>4</sub> receptor allele or mammalian 5-HT<sub>4</sub> receptor allele.

This invention provides a method of preparing the isolated 5-HT<sub>4</sub> receptor which comprises inducing cells to express receptor, recovering the receptor from the resulting cells, and purifying the receptor so recovered. An example of a 5-HT<sub>4</sub> receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 5. For example, cells can be induced to express receptors by exposure to substances such as hormones. The cells can then be homogenized and the receptor isolated from the homogenate using an affinity column comprising, for example serotonin or another substance which is known to bind to the 5-HT<sub>4</sub> receptor. The resulting fractions can then be purified by contacting them with an ion exchange column, and determining which fraction contains 5-HT<sub>4</sub> receptor activity or binds anti-receptor antibodies.

This invention provides a method of preparing an isolated human 5-HT<sub>4</sub> receptor which comprises inserting nucleic acid encoding the human 5-HT<sub>4</sub> receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered. An example of an isolated human 5-HT<sub>4</sub> receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence

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shown in Figure 5 (SEQ ID NOS. 5 and 6). This invention provides a method of preparing an isolated mammalian 5-HT<sub>4</sub> receptor which comprises inserting nucleic acid encoding the mammalian 5-HT<sub>4</sub> receptor in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the receptor produced by the resulting cell, and purifying the receptor so recovered. An example of an isolated mammalian 5-HT<sub>4</sub> receptor is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figures 1 and 2 (SEQ ID NOS. 1-2 and Seq I. D. Nos. 3-4, respectively). These methods for preparing 5-HT<sub>4</sub> receptor uses recombinant DNA technology methods well known in the art. For example, isolated nucleic acid encoding 5-HT<sub>4</sub> receptor is inserted in a suitable vector, such as an expression vector. A suitable host cell, such as a bacterial cell, insect cell, or a eukaryotic cell such as a yeast cell, is transfected with the vector. 5-HT<sub>4</sub> receptor is isolated from the culture medium by affinity purification or by chromatography or other methods well known in the art.

This invention provides a method for determining whether a compound not known to be capable of specifically binding to a human 5-HT<sub>4</sub> receptor can specifically bind to the human 5-HT<sub>4</sub> receptor, which comprises contacting a mammalian cell comprising a plasmid adapted for expression in a mammalian cell which plasmid further comprises a DNA which expresses a human 5-HT<sub>4</sub> receptor on the cell's surface with the compound under conditions permitting binding of ligands known to bind to a human 5-HT<sub>4</sub> receptor, detecting the presence of any compound bound to the human 5-HT<sub>4</sub> receptor, the presence of bound compound indicating that the compound is capable of specifically binding to the human 5-HT<sub>4</sub> receptor.

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This invention provides a method for determining whether a compound not known to be capable of specifically binding to a mammalian 5-HT<sub>4</sub> receptor can specifically bind to the mammalian 5-HT<sub>4</sub> receptor, which comprises contacting a mammalian cell comprising a plasmid adapted for expression in a mammalian cell which plasmid further comprises a DNA which expresses a mammalian 5-HT<sub>4</sub> receptor on the cell's surface with the compound under conditions permitting binding of ligands known to bind to a mammalian 5-HT<sub>4</sub> receptor, detecting the presence of any compound bound to the human 5-HT<sub>4</sub> receptor, the presence of bound compound indicating that the compound is capable of specifically binding to the mammalian 5-HT<sub>4</sub> receptor.

This invention provides a method for identifying a compound which is not known to be capable of binding to a human 5-HT<sub>4</sub> receptor can functionally activate the human 5-HT<sub>4</sub> receptor on the surface of a mammalian cell or prevent a ligand which does so, which comprises contacting the mammalian cell which cell comprises a plasmid adapted for expression in the mammalian cell such plasmid further comprising DNA which expresses the human 5-HT<sub>4</sub> receptor on the surface of the mammalian cell with the compound, determining whether the compound activates the human 5-HT<sub>4</sub> receptor or prevents a ligand which does so, and thereby identifying the compound as a compound which binds to and functionally activates the human 5-HT<sub>4</sub> receptor or prevents the functional activation of the human 5-HT<sub>4</sub> receptor by a ligand which does so.  
The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 5 (SEQ ID No. 5).

This invention provides a method for identifying a compound which is not known to be capable of binding to

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a mammalian 5-HT<sub>4</sub> receptor can functionally activate the mammalian 5-HT<sub>4</sub> receptor on the surface of a mammalian cell or prevent a ligand which does so, which comprises contacting the mammalian cell which cell comprises a plasmid adapted for expression in the mammalian cell such plasmid further comprising DNA which expresses the mammalian 5-HT<sub>4</sub> receptor on the surface of the mammalian cell with the compound, determining whether the compound activates the mammalian 5-HT<sub>4</sub> receptor or prevents a ligand which does so, and thereby identifying the compound as a compound which binds to and functionally activates the mammalian 5-HT<sub>4</sub> receptor or prevents the functional activation of the mammalian 5-HT<sub>4</sub> receptor by a ligand which does so. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figures 1 and 2 (SEQ ID NOS. 1 and 3).

The activation or blockade of the functional response is detected by means of a bioassay from the mammalian cell such as a second messenger response, and thereby determining whether the compound activates or prevents the activation of the 5-HT<sub>4</sub> receptor functional output. Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is an LM (tk-) cell. Another example of a non-neuronal mammalian cell to be used for functional assays is a murine fibroblast cell line, specifically the NIH3T3 cell. The preferred method for determining whether a compound is capable of binding to the 5-HT<sub>4</sub> receptor comprises contacting a transfected nonneuronal mammalian cell (i.e. a cell that does not naturally express any type of 5-HT or G-protein coupled receptor, thus will only express such a receptor if it is transfected into the cell) expressing a 5-HT<sub>4</sub> receptor on its surface, or contacting a membrane preparation derived from such a transfected cell, with the compound

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under conditions which are known to prevail, and thus to be associated with, *in vivo* binding of ligands to a 5-HT<sub>4</sub> receptor, detecting the presence of any of the compound being tested bound to the 5-HT<sub>4</sub> receptor on the surface of the cell, and thereby determining whether the compound binds to, and activates or prevents the activation of the 5-HT<sub>4</sub> receptor. This response system is obtained by transfection of isolated DNA into a suitable host cell containing the desired second messenger system such as phosphoinositide hydrolysis, adenylate cyclase, guanylate cyclase or ion channels. Such a host system is isolated from pre-existing cell lines, or can be generated by inserting appropriate components of second messenger systems into existing cell lines. Such a transfection system provides a complete response system for investigation or assay of the activity of human 5-HT<sub>4</sub> receptor with compounds as described above.

Transfection systems are useful as living cell cultures for competitive binding assays between known or candidate drugs and ligands which bind to the receptor and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the receptor isolated from transfected cells are also useful for these competitive binding assays. Functional assays of second messenger systems or their sequelae in transfection systems act as assays for binding affinity and efficacy in the activation of receptor function. A transfection system constitutes a "drug discovery system" useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate or inhibit the natural functions of the human 5-HT<sub>4</sub> receptor. The transfection system is also useful for determining the affinity and efficacy of known drugs at

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human 5-HT<sub>4</sub> receptor sites.

This invention also provides a method of screening compounds to identify drugs which interact with, and specifically bind to, a human 5-HT<sub>4</sub> receptor on the surface of a cell, which comprises contacting a mammalian cell which comprises a plasmid adapted for expression in a mammalian cell which plasmid further comprises DNA which expresses a human 5-HT<sub>4</sub> receptor on the cell's surface with a plurality of compounds, determining those compounds which bind to the human 5-HT<sub>4</sub> receptor expressed on the cell surface of the mammalian cell, and thereby identifying compounds which interact with, and specifically bind to, the human 5-HT<sub>4</sub> receptor. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 5 (SEQ ID NO. 5). This invention also provides a method of screening compounds to identify drugs which interact with, and specifically bind to, a mammalian 5-HT<sub>4</sub> receptor on the surface of a cell, which comprises contacting a mammalian cell which comprises a plasmid adapted for expression in a mammalian cell which plasmid further comprises DNA which expresses a mammalian 5-HT<sub>4</sub> receptor on the cell's surface with a plurality of compounds, determining those compounds which bind to the mammalian 5-HT<sub>4</sub> receptor expressed on the cell surface of the mammalian cell, and thereby identifying compounds which interact with, and specifically bind to, the mammalian 5-HT<sub>4</sub> receptor. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figures 1 and 3 (SEQ ID NOS. 1 and 2). Various methods of detection may be employed. The compounds may be "labeled" by association with a detectable marker substance (e.g., radiolabel or a non-isotopic label such as biotin). Preferably, the mammalian cell is nonneuronal in origin. An example of

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a nonneuronal mammalian cell is a Cos-7 cell. Drug candidates are identified by choosing chemical compounds which bind with high affinity to the expressed 5-HT<sub>4</sub> receptor protein in transfected cells, using radioligand binding methods well known in the art, examples of which are shown in the binding assays described herein. Drug candidates are also screened for selectivity by identifying compounds which bind with high affinity to one particular receptor but do not bind with high affinity to any other receptor subtypes or to any other known receptor. Because selective, high affinity compounds interact primarily with the target 5-HT<sub>4</sub> receptor site after administration to the patient, the chances of producing a drug with unwanted side effects are minimized by this approach.

This invention provides a pharmaceutical composition comprising a drug identified by the method described above and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. Once the candidate drug has been shown to be adequately bioavailable following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models, the drug may be administered to patients by that route of administration determined to make the drug bioavailable, in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

Applicants have identified a novel 5-HT receptor subtype protein, designated 5-HT<sub>4</sub>, and have described methods for the identification of pharmacological compounds for therapeutic treatments. Pharmacological compounds which are directed against specific receptor subtypes provide effective new therapies with minimal side effects.

Elucidation of the molecular structures of the neuronal serotonin receptors is an important step in the understanding of serotonergic neurotransmission. This disclosure reports the isolation and amino acid sequence of a novel cDNA which encodes a human 5-HT<sub>4</sub> receptor. This disclosure reports the isolation, amino acid sequence, and functional expression of a two novel cDNAs which encode mammalian 5-HT<sub>4</sub> receptors. The identification of 5-HT receptor subtypes play a pivotal role in elucidating the molecular mechanisms underlying serotonergic transmission, and should also aid in the development of novel therapeutic agents.

A complementary DNA clone (designated pBluescript-hS10) encoding a serotonin receptor subtype, 5-HT<sub>4</sub>, has been isolated from human brain, human heart and human retina. Additionally, two complementary DNA clones encoding the serotonin 5-HT<sub>4</sub> receptor subtype have been isolated from mammalian brain and their functional properties have been examined in mammalian cells. Analysis of 5-HT<sub>4</sub> structure and function provides a model for the development of drugs useful for the treatment of gastrointestinal conditions including irritable bowel disease, postoperative ileus, diabetic gastroparesis, emesis, achalasia, hiatal hernia, and esophageal spasm. In addition, 5-HT<sub>4</sub> receptors have been described functionally in the heart (Kaumann,

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1992), adrenal (Lefebvre et al., 1992), and bladder (Corsi et al., 1991) indicating possible roles in cardiac rate and force of contraction, endocrine control of cortisol secretion, and urinary incontinence or spasticity. 5-HT<sub>4</sub> receptors have also been described in the brain, particularly in areas such as the hippocampus, in which we have localized the gene encoding 5-HT<sub>4</sub> receptors (S10-95), indicating a potential role in cognitive enhancement (Bockaert et al., 1992).

This invention identifies a mammalian serotonin receptor, its amino acid sequence, and its mammalian gene, the activation of which is coupled to activation of adenylate cyclase. The information and experimental tools provided by this discovery are useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this receptor protein, its associated mRNA molecule or its associated genomic DNA. The information and experimental tools provided by this discovery will be useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new serotonin receptor subtype, its associated mRNA molecule, or its associated genomic DNA.

Specifically, this invention relates to the isolation of human cDNA clone and mammalian cDNA clones encoding a new serotonin receptor, designated 5-HT<sub>4</sub>. In addition, the mammalian 5-HT<sub>4</sub> receptors have been expressed in COS-7 cells by transfecting the cells with the plasmids pcEXV-S10-87 and pcEXV-S10-95. The pharmacological binding properties of the encoded 5-HT<sub>4</sub> receptor have been determined, and the binding properties classify this receptor as a novel serotonin receptor. Mammalian cell lines expressing the mammalian 5-HT<sub>4</sub> receptor on the cell surface have been constructed, thus establishing the first well-defined,

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cultured cell lines with which to study the novel 5-HT<sub>6</sub> receptor.

The invention will be better understood by reference to  
5 the Experimental Details which follow, but those  
skilled in the art will readily appreciate that the  
specific experiments detailed are only illustrative,  
and are not meant to limit the invention as described  
herein, which is defined by the claims which follow  
10 thereafter.

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Methods and Materials

PCR Amplification: The third (III) and fifth (V) transmembrane (TM) domains of the following receptors were aligned and used to synthesize a pair of degenerate primers: 5-HT<sub>1A</sub>, 5-HT<sub>1C</sub>, 5-HT<sub>2</sub> and the 5-HT<sub>10a/b</sub> receptors. Primers 3.17 and 5.5 ([5'-TGGAAATTCTG(C/T)G(C/T)IAT(A/C/T)(G/T)CICGTGGA(C/T)(A/C)G(C/G)TA-3'] (SEQ ID NO. 9), [5'-CATIA(G/C/A)I(G/A)IIA(G/A)IGG(T/G/A/)AT(G/A)(T/A)A(G/A)AAIGC-3']) (SEQ ID No. 10) were used to amplify 5 µg of poly (A+) RNA from rat brain that was reverse transcribed by avian myeloblastosis virus reverse transcriptase (AMV). PCR was performed on single-stranded cDNA under the following conditions: 94°C for 1 min, 50°C for 2 min and 72°C for 3 min for 40 cycles. Following PCR, 90 µl of the reaction was phenol:chloroform extracted and precipitated; 10 µl was visualized on a gel using ethidium bromide staining. After precipitation the sample was treated with T4 DNA polymerase and digested with EcoR1 prior to separation on a 1% agarose gel. The DNA fragments (200 to 400 base pairs) were isolated from the gel, kinased and cloned into pBluescript. Recombinant clones were analyzed by sequencing. One fragment 270 base pairs in length, named S10, was identified. This sequence contained a "TM IV" like domain and represented a potentially new serotonin receptor. The corresponding full length cDNA was isolated from a rat brain cDNA library.

Rat PCR primers (from TM3 to TM7) were used to amplify single-stranded cDNA prepared from human heart, brain and retina, as described above. Those human PCR DNA fragments were subcloned in pBluescript and sequenced.

**cDNA Library Construction, Screening and Sequencing:**

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Rat brains were dissected from adult male CD rats (Charles River Laboratories) and total RNA was prepared by the guanidine thiocyanate method (Chirgwin, J.W. et al.; 1979). Poly A<sup>+</sup>RNA was purified with a Fast track kit (Invitrogen Corp., San Diego, CA). Double stranded (DS) cDNA was synthesized from 5 µg of poly A<sup>+</sup>RNA according to Gubler and Hoffman (Gubler, U. and B. J. Hoffman, 1983). The resulting DS cDNA was ligated to BstXI/EcoRI adaptors (Invitrogen Corp.), the excess of adaptors was removed by chromatography on Sepharose CL 4B (Pharmacia LKB) and the DNA was then size selected on a Gen-Pak Fax HPLC column (Zhao, D. et al., 1992) (Waters, Millipore Corp., Milford, MA). High molecular weight fractions were ligated in pCDM8 cut by BstXI (Invitrogen Corp.). The ligated DNA was electroporated in E.Coli MC 1061 (Gene Pulser, Biorad). A total of  $20 \times 10^6$  independent clones with an insert mean size of 1.9 kb could be generated. Before amplification, the library was divided into pools of 2.5 to  $5 \times 10^4$  independent clones. After 18 hours amplification, the pools were stored at -85°C in 20% glycerol.

100 pools of the cDNA library, representing  $3.2 \times 10^6$  primary clones, were screened using exact PCR primers derived from the S10 PCR clone sequence. 1 µl ( $4 \times 10^6$  bacteria) of each amplified pool was subjected directly to 40 cycles of PCR and the resulting products analyzed by agarose gel electrophoresis and Southern blotting. Two out of four positive pools were analyzed further and by sib selection and plating out, two individual full length cDNA clones, S10-87 and S10-95, were isolated. DS-DNA was sequenced with a sequanase kit (US Biochemical, Cleveland, OH) according to the manufacturer. Nucleotide and peptide sequences analysis were performed with GCG programs.

Genomic Cloning and Sequencing: A human fibroblast

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genomic library in  $\lambda$  dash II ( $\approx 1.5 \times 10^6$  total recombinants; Stratagene, LaJolla, CA) was screened using a 45 nt. oligonucleotide probe derived from the rat S10-87 receptor gene, designed in the 3' end of the carboxyl terminal tail (from the anti-sense strand [n u c l e o t i d e    1 2 2 0 - 1 2 6 4 ] , 5' TCAAAAGCATGATTCCAGGGACTCTGGGTCTTGTGTATGGG CAA 3' (SEQ ID No. 11) (see Fig.1). The oligomer was labeled with [ $^{32}$ P] $\gamma$ ATP by using polynucleotide kinase. Hybridization was performed at medium stringency conditions: 45°C. in a solution containing 37.5% formamide, 5x SSC (1X SSC is 0.15M sodium chloride, 0.015M sodium citrate), 1x Denhardt's solution (0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin), and 200  $\mu$ g/ $\mu$ l sonicated salmon sperm DNA. The filters were washed at 45°C. in 0.1x SSC containing 0.1% sodium dodecyl sulfate and exposed at -70°C. to Kodak XAR film in the presence of an intensifying screen. Lambda phage clones hybridizing with the probe were plaque purified and DNA was prepared for Southern blot analysis (Southern, 1975; Sambrook et al., 1989). A 900 bp Hind2/SstI hybridizing fragment was subcloned into pUC18 (Pharmacia, Piscataway, NJ)). Nucleotide sequence analysis was accomplished by the Sanger dideoxy nucleotide chain termination method (Sanger et al., 1977) on denatured double-stranded plasmid templates, using Sequenase (US Biochemical Corp., Cleveland, OH).

30      **PCR amplification of a partial length human S10-87 cDNA clone:**

The 900 bp Hind2/SstI fragment contained sequence encoding the human S10-87 carboxy terminal tail, including the stop codon. This sequence was used to generate a 25 mer (reverse primer) containing the stop codon: 5' CCTCAATCAGAAGCATGATTCCAGG 3' (SEQ ID No. 12). As a forward primer we used the 5' end of the human PCR

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fragment previously identified (figure 6): 5' TTGGTCTATAGGAACAAGATGACCC 3' (SEQ ID No. 13). These human PCR primers were used to amplify single stranded cDNA prepared from human brain as previously described. 5 The amplified DNA was subcloned and sequenced as described above.

Isolation of the full length human S10-95 cDNA clone: 10 20 pools of a human hippocampal cDNA library (3kb average size insert, in pcEXV-3) representing 10<sup>6</sup> independent clones were screened by PCR with TM4-TM6 primers as previously described. Five positive pools were identified. one of those pools was analyzed further and by sib selection a 5kb cDNA clone, CG-17, 15 was isolated. Double Stranded-DNA was sequenced as described above. Nucleotide and peptide sequence analysis were performed with the Genetics Computer Group sequence analysis software package.

20 DNA transfection: The full coding region of S10-87 (clone CG-5) and S10-95 (clones CG-6 and CG-17) were subcloned in the correct orientation in the mammalian expression vectors pCDNA1-Amp (Invitrogen Corp.), and pcEXV-3 (Miller, J. and R. N. Germain, 1986) (CG-7 and CG-8 respectively). For transient expression, Cos-7 25 cells were transfected by the DEAE-Dextran method, using 1 µg of DNA /10<sup>6</sup> cells (Warden, D. and H.V. Thorne, 1968).

30 Membrane Preparation: Membranes were prepared from transiently transfected COS-7 cells which were grown to 100% confluence. The cells were washed twice with phosphate-buffered saline, scraped from the culture dishes into 5 ml of ice-cold phosphate-buffered saline, and centrifuged at 200 x g for 5 min at 4°. The pellet 35 was resuspended in 2.5 ml of ice-cold Tris buffer (20 mM Tris -HCl, pH 7.4 at 23°, 5 mM EDTA), and

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homogenized by a Wheaton tissue grinder. The lysate was subsequently centrifuged at 200 x g for 5 min at 4°. The pellet large fragments which were discarded. The supernatant was collected and centrifuged at 40,000 x g for 20 min at 4°. The pellet resulting from this centrifugation was washed once in ice-cold Tris wash buffer and finally resuspended in a final buffer containing 50 mM Tris-HCl and 0.5 mM EDTA, pH 7.4 at 23°. Membrane preparations were kept on ice and utilized within two hr for the radioligand binding assays. Protein concentrations were determined by the method of Bradford (1976) using bovine serum albumin as the standard.

Radioligand Binding: [<sup>3</sup>H]5-HT binding was performed using slight modifications of the 5-HT<sub>1D</sub> assay conditions reported by Herrick-Davis and Titeler (1989) with the omission of masking ligands. Radioligand binding studies were achieved at 37° C in a total volume of 250 µl of buffer (50 mM Tris, 10 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 10 µM pargyline, 0.1 % ascorbate, pH 7.4 at 37° C) in 96 well microtiter plates. Saturation studies were conducted using [<sup>3</sup>H]5-HT at 10 different concentrations ranging from 1.0 nM to 100 nM. Displacement studies were performed using 10 nM [<sup>3</sup>H]5-HT. The binding profile of drugs in competition experiments was established using 7 concentrations of compound. Incubation times were 30 min for both saturation and displacement studies. Nonspecific binding was defined in the presence of 10 µM 5-HT. Binding was initiated by the addition of 50 µl membrane homogenates (10-20 µg). The reaction was terminated by rapid filtration through presoaked (0.5% polyethyleneimine) filters using 48R Cell Brandel Harvester (Gaithersburg, MD). Subsequently, filters were washed for 5 sec with ice cold buffer (50 mM Tris HCl, pH 7.4 at 4° C), dried and placed into vials

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containing 2.5 ml of Readi-Safe (Beckman, Fullerton, CA), and radioactivity was measured using a Beckman LS 6500C liquid scintillation counter. The efficiency of counting of [<sup>3</sup>H]5-HT averaged between 45-50%. Binding data were analyzed by computer-assisted nonlinear regression analysis (Accufit and Accucomp, Lunden Software, Chagrin Falls, OH). IC<sub>50</sub> values were converted to K<sub>i</sub> values using the Cheng-Prusoff equation (1973).

[<sup>3</sup>H]GR113808 binding was performed using slight modifications of the method of Waeber et al., 1993. Radioligand binding studies were achieved at 37°C in a total volume of 250 µl of buffer (50 mM Tris, 10 µM, 0.01% ascorbate, pH 7.4 at 37°C) in 96 well microtiter plates. Saturation studies were conducted using [<sup>3</sup>H]GR113808 at 10-12 different concentrations ranging from 0.005-2.5 nM. Displacement studies were performed using 0.2-0.4 nM [<sup>3</sup>H]GR113808. The binding profile of drugs in competition experiments was established using 10-12 concentrations of compound. Incubation times were 30 min for both saturation and displacement studies. Nonspecific binding was defined in the presence of 50 µM 5-HT. Binding was initiated and terminated as described for [<sup>3</sup>H]5-HT binding (see above). Radioactivity was measured and data were analyzed as described above for [<sup>3</sup>H]5-HT.

Measurement of cAMP Formation: The transiently transfected Cos-7 cells were incubated in Dulbecco's modified Eagle's medium, 5 mM theophylline, 10 mM Hepes (4-[2-Hydroxyethyl]-1-piperazineethanesulfonic acid), 10 µM pargyline, and/or appropriate concentrations of antagonists for 20 minutes at 37°C, 5% CO<sub>2</sub>. Serotonin or other agonists in the presence or absence of forskolin (FSK) (10 µM) were then added at appropriate concentrations and incubated for an additional 10

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minutes at 37°C, 5% CO<sub>2</sub>. The media was aspirated and the reaction stopped by the addition of 100 mM HCl. The plates were stored at 4°C for 15 minutes, centrifuged for 5 minutes, 500 x g to pellet cellular debris, and the supernatant aliquotted and stored at -20°C prior to assessment of cAMP formation by radioimmunoassay (cAMP Radioimmunoassay kit, Advanced Magnetics, Cambridge, MA). Radioactivity was quantitated using a Packard COBRA Auto Gamma Counter equipped with data reduction software. Functional data was fitted to a four parameter logistic equation to obtain response parameters (EC<sub>50</sub>, E<sub>max</sub>, nH; Inplot, GraphPad, San Diego, CA).

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Drugs: [<sup>3</sup>H]5-HT (specific activity = 22.7 Ci/mmol) was obtained from New England Nuclear, Boston, MA. [<sup>3</sup>H]GR113808 (specific activity = 82 Ci/mmol) was obtained from Amersham International (Arlington Hts., IL). All other chemicals were obtained from commercial sources and were of the highest grade purity available.

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Experimental Results

A 270 bp DNA fragment (S10) was identified when rat brain cDNA was used as template in a PCR amplification with two degenerate oligonucleotide primers derived from well conserved regions among several serotonin receptors, in the third and fifth putative transmembrane domains. The peptide sequence corresponding to this S10 PCR clone contained a "transmembrane IV like" domain. Since we used "serotonin receptor specific" PCR primers, this S10 clone represented a potentially new serotonin receptor. The corresponding full length cDNA was isolated from a rat brain cDNA library. Since five amplified commercial phage cDNA libraries turned out to be negative, we split the plasmid cDNA library into small pools of 2.5 to  $5 \times 10^4$  independent clones before amplification to avoid a potential growth bias against the S10 cDNA clone. By direct PCR analysis of bacterial pools, subsequent sib selection and standard filter hybridization, two cDNA clones were identified, S10-87 (5.5 kb) and S10-95 (4.5 kb). The nucleotide and deduced amino acid sequences are shown in Figure 1 (S10-87) and Figure 2 (S10-95). Surprisingly the peptide sequences between those two clones are only 96.7% identical, diverging in the second half of the carboxy terminus tails, downstream of position 359 (Figure 3). In addition, the entire 3' untranslated regions are totally divergent. The longest open reading frame for S10-87 encodes a protein of 387 amino acids and 406 amino acids for S10-95. The hydrophobicity plot displayed seven hydrophobic, putative membrane spanning regions which when compared to other G protein-coupled receptors did not show any significant high homologies, even to other serotonin receptors (Table 1 and figure 4). It is interesting to note that the highest homology, overall or restricted

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to the 7 TM region, is exhibited by the human histamine H<sub>2</sub> receptor, which like the 5-HT<sub>4</sub> receptor, is coupled to stimulation of cAMP.

5 Both S10-87 and S10-95 proteins carry 4 potential N-glycosylation sites in positions 7, 180, 316, and 352. They also possess 3 potential phosphorylation sites for protein kinase C in positions 218, 248, 318 and 4 potential phosphorylation sites for casein kinase II in  
10 positions 9, 97, 218 and 288. A potential palmitoylation site is present in both clones at the cysteine found in position 329. A large number of G protein-coupled receptors carry a cysteine in the same position and O'Dowd et al. have speculated that it  
15 plays an important role in the functional coupling of the human β<sub>2</sub>-adrenergic receptor. In addition, clone S10-95 carries one more potential phosphorylation site for protein kinase C at position 400. This additional phosphorylation site could lead to differential functional coupling between the S10-87 and S10-95 receptors.  
20

Since we isolated two different S10 cDNA clones by screening a library made from an entire brain, we  
25 checked for differential expression in seven different parts of the brain by PCR amplification using pairs of primers specific for each clone. The results are summarized in table 2. Clone S10-95 seems to be transcribed everywhere in the rat brain except in cerebellum. Clone S10-87 is only expressed in striatum. It remains to be determined if only one or  
30 both receptors are expressed in rat cortex.  
35

The partial human S10-87 nucleotide (Fig 11A) and deduced amino acid sequences (Fig 11B) are shown. The sequences are highly similar to the rat S10-87 homolog, 90.8 % at the nucleotide level and 93.8 % at the amino

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acid level (figures 12 and 13 respectively).

The full length human S10-95 nucleotide (Fig 14A) and deduced amino acid sequences (Fig 14B) are shown.

5      Compared to the rat S10-95 sequence, it shows 90.7 % identity at the nucleotide level and 91.8 % identity at the amino acid level (figures 15 and 16 respectively). The human S10-95 nucleotide sequence contains one nucleotide insertion in position 1159.

10     This insertion creates a frame shift and introduces a stop codon in the reading frame 16 nucleotides downstream. The protein motifs are highly conserved between the rat and human homologs except for a casein kinase II potential phosphorylation site in position 288 which is lost in both human receptors. The human homologs both carry a potential cAMP/cGMP phosphorylation site in position 338 in their carboxy terminal tail which is absent in the rat homologs. A comparison of the amino acid sequence between the human and the rat S10-95 clones beginning from the initiating methionine and ending with the stop codon of the human S10-95 clone, reveals 31 amino acid changes of which 11 are non conservative, including 2 in TM1, 1 in TM2 and 1 in TM4. Due to the nucleotide insertion and the corresponding frame shift described above, the carboxy terminal tail of the human S10-95 receptor is 16 amino acid shorter than its rat homolog.

15     Identical to the rat homologs, both human clones are identical in the loops and transmembrane regions, differing only in the second half of their carboxy terminal tail (fig 17, nucleic acid sequence; fig 18, aa sequence).

20     The human PCR cDNA fragments (TM-3 to TM-7) are 100% identical between heart, brain and retina. The nucleotide and deduced amino acid sequences are shown

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in figure 5. The human sequence shows 90.7% homology with the rat nucleotide sequence (Figure 6) and 92.3% homology (Figure 7) with the rat amino acid sequence.

5      The genes encoding the rat S10-87 and S10-95 receptors were transiently expressed in Cos-7 cells for pharmacological evaluation. Initial experiments using 5 nM [<sup>3</sup>H]5-HT indicated that both S10-87 and S10-95 were serotonergic sites as demonstrated by the degree of specific binding and density of sites expressed in the transfected cells when compared against the mock transfected controls. Saturation analysis of S10-87 (CG-7) was performed using 10 concentrations of [<sup>3</sup>H]5-HT (1-100 nM) and yielded a B<sub>max</sub> of 1,938 ± 399 fmol/mg of protein and a K<sub>d</sub> for [<sup>3</sup>H]5-HT of 7.87 ± 0.06 nM. The degree of specific binding at concentrations of [<sup>3</sup>H]5-HT close to the K<sub>d</sub> ranged from 70-84% throughout the experimental series (including saturation and competition studies). Although the use of [<sup>3</sup>H]5-HT as a radioligand to label 5-HT<sub>2</sub> receptors in brain tissue is not efficient due to the nonselectivity of the ligand, it became clear in the present studies using a homogeneous receptor population that [<sup>3</sup>H]5-HT would label this particular receptor. In fact, [<sup>3</sup>H]5-HT appears to be labelling the high affinity state of the 5-HT<sub>2</sub> receptor which is not unusual for the conditions upon which this receptor has been studied. Similar results using an agonist radioligand have been previously reported for the cloned 5-HT<sub>2</sub> receptor (Branchek et al., 1990).

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35      A pharmacological binding profile of S10-87 and S10-95 (CG-7 and CG-8) was performed and demonstrated that this novel receptor was similar to the 5-HT<sub>2</sub> receptor as defined by functional assays in the literature (Bockaert et al., 1992). This is clearly shown in table 3 where the binding affinities of various

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serotonergic agonists are displayed for S10. Notably, 5-HT and the tryptamine derivative 5-methoxytryptamine possessed high affinity. Furthermore, as previously reported for the 5-HT<sub>4</sub> receptor, benzamide derivatives including cisapride, BRL 24924 and zacopride bound with fairly high affinity to receptors expressed from the S10 gene. ICS 205930, a tropanyl-indole derivative, which has been reported to be an antagonist at both 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors (Bockaert et al., 1992), also bound to these receptor sites. Compounds such as 8-hydroxy-2-(di-n-propylamino)tetralin, ketanserin, sumatriptan and 5-carboxyamidotryptamine were of low affinity having K<sub>i</sub> values estimated to be greater than 1 μM. This data would rule out S10 from belonging to other serotonergic receptor subfamilies such as 5-HT<sub>1</sub> and 5-HT<sub>2</sub>. Taken together, the complete pharmacological profile also differentiates S10 from the related subtype 5-HT<sub>4B</sub> (U.S. Serial No., 971,960, filed, November 3, 1992, copending). Although some of the drugs tested also have good affinity for 5-HT<sub>3</sub> receptors, S10 is clearly a 5-HT<sub>4</sub> receptor based upon the binding data and the functional data demonstrating a positive-coupling to adenylate cyclase. Finally, a correlation plot for the binding affinities of 5-HT, cisapride, BRL 24924, zacopride, and ICS 205930 against their functional responses in adenylate cyclase assays from cultured mouse collicular neurons (Dumuis et al., 1989) yielded a correlation coefficient of 0.96 (Figure 8). Thus, the rank order of potency for these key compounds also provides conclusive evidence that S10 encodes a 5-HT<sub>4</sub> receptor.

To examine the ability of S10 clone to couple to adenylate cyclase, Cos-7 cells transiently expressing S10 were tested for the ability to exhibit an increase in basal cAMP release or a decrease in FSK-stimulated cAMP response. 5-HT (1 μM) had no effect on either

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basal or FSK-stimulated adenylate cyclase activity in untransfected or mock-transfected Cos-7 cells (data not shown), indicating that endogenous cyclase-coupled serotonin receptors are not present in untransfected cells. Preliminary studies were carried out by adding only one dose of various drugs in triplicate. Addition of 5-HT (1  $\mu$ M) to this system caused stimulation of basal cAMP release (CG-7= 0.020  $\pm$  0.002; CG-8= 0.023  $\pm$  0.004 pmol/ml/10min) by about 30 fold for each clone; no inhibition of either the basal or FSK-stimulated cAMP release was observed. On the contrary, addition of 10  $\mu$ M FSK together with 1  $\mu$ M 5-HT stimulated cAMP accumulation about 10-fold more than either agent alone (data not shown). For various compounds, full dose-response curves were determined for both clones and the data are summarized in table 4. 5-HT caused a concentration-dependent stimulation of basal adenylate cyclase activity with mean EC<sub>50</sub>s of 26  $\pm$  3 and 51  $\pm$  7 nM and E<sub>max</sub>s of 2,107 and 2,598 % basal cAMP release for CG-7 and CG-8 respectively (Figures 9 and 10). Among the tryptamine derivatives tested, 5-MeOT was approximately equipotent with 5-HT in both clones, whereas  $\alpha$ -Me-5-HT and 5-CT were about 10 and 200 times respectively less potent than 5-HT at CG-7. On the other hand, the latter two compounds displayed approximately 20 and 30 fold lower affinity than 5-HT respectively for CG-8. The 2-methoxy-4-amino-5-chloro-substituted benzamides (cisapride, BRL-24924 and zacopride) were less potent agonists than 5-HT in stimulating basal cAMP release and displayed different rank order of potency for CG-7 and CG-8. As indicated in table 4 using CG-7, cisapride, BRL-24924 and zacopride exhibited approximately 10, 30 and 100 fold lower potency than 5-HT respectively, whereas at CG-8 these compounds had almost equal affinity. Thus, although not different in binding properties, these subtle differences in affinity in functional assays f

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the two "variants" (CG-7 and CG-8) indicate the potential to develop separate therapeutic entities for each separate target. All the agonists tested acted as full agonists with the exception of cisapride, BRL-24924 and zacopride, which also displayed antagonist activity and were therefore partial agonists at both clones, with intrinsic activities ranging between 0.85 and 1.4 (Table 4). ICS-205-930 (100  $\mu$ M) had similar effect at the two clones and was found to be a silent antagonist causing parallel dextral shifts in the concentration effect curve of 5-HT without altering the maximum response significantly. The estimated  $K_b$  value for ICS-205-930 was not significantly different between the two clones (CG-7= 962  $\pm$  244 nM; CG-8= 607  $\pm$  30 nM). Responses to 5-HT were not affected by spiperone or methiothepin (10  $\mu$ M) in either clone.

Saturation analysis of rat 5-HT<sub>4A</sub> S10-87 (CG-7) and S10-95 (CG-8) clones and human 5-HT<sub>4A</sub> clone CG-17 were performed using 10-12 concentrations of [<sup>3</sup>H]GR113808 (0.005-2.5 nM) and revealed a single saturable site of high affinity for both clones (CG-7:  $K_d$  = 0.74 nM,  $B_{max}$  = 5.7 pmol/mg membrane protein; CG-8:  $K_d$  = 1.0 nM,  $B_{max}$  = 3.7 pmol/mg membrane protein; CG-17:  $K_d$  = 0.20 nM,  $B_{max}$  = 1.8 pmol/mg membrane protein). These preliminary data indicate that although the rat clones (CG-7 and CG-8) have similar affinity for the antagonist [<sup>3</sup>H]GR113808, the human clone (CG-17) displays approximately 5-fold higher affinity than the rat clones for this ligand. For all three clones nonspecific binding increased linearly with increasing ligand concentration. The degree of specific binding at concentrations of [<sup>3</sup>H]GR113808 (0.4-0.5 nM) ranged from 80-90%.

The pharmacological binding profile of S10-87 and S10-95 (CG-7, CG-8, respectively) was investigated in

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displacement studies using [<sup>3</sup>H]GR113808 and/or [<sup>3</sup>H]5-HT. In order to compare CG-17 pharmacology with that previously obtained for the rat clones, CG-7 and CG-8, displacement studies on the human CG-17 clone were performed using [<sup>3</sup>H]5-HT as the radioligand.

A range of 5-HT<sub>1</sub> receptor agonists and antagonists completely inhibited the specific binding of [<sup>3</sup>H]GR113808 on both the rat CG-7 and CG-8 clones. Affinity values and Hill slopes derived from the curves using computer analysis are presented in Table 5. As previously observed using [<sup>3</sup>H]5-HT as the radioligand, the rat CG-7 and CG-8 receptors had very similar pharmacology. Of the agonists tested, only those active in 5-HT<sub>1</sub>, containing preparations (5-HT and 5-MeOT) potently inhibited [<sup>3</sup>H]GR113808. By contrast, agonists for other 5-HT receptors, for example 5-HT<sub>1A</sub> receptor agonist, 8-OH-DPAT, the 5-HT<sub>1B</sub> receptor agonist, sumatriptan, the 5-HT<sub>2</sub> receptor antagonist, ketanserin, had no effect on [<sup>3</sup>H]GR113808 binding at concentrations up to 1 μM. The substituted benzamides, cisapride, BRL-24924 and zacopride, partial agonists at 5-HT<sub>1</sub> receptor all potently inhibited [<sup>3</sup>H]GR113808 binding. Specific [<sup>3</sup>H]GR113808 binding was also inhibited by the 5-HT<sub>2</sub> receptor antagonist ICS-205930.

For both the rat CG-7 and CG-8 clones, Hill slopes for the inhibition of [<sup>3</sup>H]GR113808 binding by 5-HT<sub>1</sub> receptor agonists but not the antagonist, ICS-205930, were shallow in the absence of Gpp(NH) with the exception of 5-CT, and α-Me-5-HT. For agonists that had shallow displacement curves, the binding was resolved into high and low affinity components and these are summarized in Table 5. The K<sub>i</sub> values obtained for the high affinity state of the receptor using [<sup>3</sup>H]GR113808 as the radioligand were compatible with the K<sub>i</sub> values obtained previously using [<sup>3</sup>H]5-HT as the radioligand which

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labels the high affinity state of the receptor (Table 5 and 6). Some differences were observed for the  $K_i$  values of high affinity state of CG-7 compared to CG-8 (Table 3) and their NH values. For example, although there were no differences in the  $K_i$  values of CG-7 and CG-8, the displacement curve obtained for 5-MeOT using CG-8 clone could not be resolved into two sites. Also the  $K_i$  value obtained for the high affinity state of CG-8 using cisapride was approximately 3-fold lower than that obtained for CG-7 previously using [ $^3$ H]5-HT to directly label the high affinity state of the receptor. We are currently investigating these differences using [ $^3$ H]5-HT to directly label the high affinity state.

In the presence of 100  $\mu$ M Gpp(NH)p, competition binding curves for the agonists displaying shallow curves in the absence of Gpp(NH)p were shifted to the right and this is exemplified for 5-HT in Fig. The Hill slopes were increased.

Preliminary results obtained with the human clone (CG-17) using [ $^3$ H]5-HT as the radioligand in displacement studies are summarized in Table 3. Similar to the rat CG-7 and CG-8 clones, 8-OH-DPAT, sumatriptan and ketanserin were inactive at the CG-17 clone for up to concentration of 1  $\mu$ M. The differences observed between the human and the rat CG-8 clones were as follows. The biggest difference was observed with  $\alpha$ -Me-5-HT which had approximately 100 fold higher affinity for the human CG-17 clone. Zacropride, 5-MeOT and cisapride had about 7-fold, 5-fold and 4-fold higher affinity, respectively for the human clone CG-17.

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DISCUSSION

W have identified two cDNA clones encoding the pharmacologically-defined 5-HT<sub>4</sub> receptor. This receptor is expressed at low levels in rat brain if we consider its frequency in the cDNA library ( $\leq 1:10^6$ ). Surprisingly, two receptors differing in their carboxy-terminus regions have been isolated. Since the 3' untranslated nucleotide sequences are also different, these two receptors could be encoded by two different genes or could arise by alternative splicing of pre-mRNA. These two receptors (S10-87 and S10-95) are differentially expressed in rat brain and the physiological relevance of the S10-87 receptor being expressed only in striatum remains to be determined.

The pharmacology binding profile and the functional coupling obtained from cells expressing S10 clones indicate that these genes both encode a pharmacologically-defined 5-HT<sub>4</sub> receptor. The cloned rat CG-7 and CG-8 genes transiently expressed in Cos-7 cells coupled to stimulation of adenylate cyclase. The magnitude of this response (~20-25 fold) was large. With the exception of 5-MeOT, agonist potencies determined from functional assays were less than expected from K<sub>i</sub> values obtained from binding assays using [<sup>3</sup>H]5-HT. Could this result be due to the possibility that the dose of [<sup>3</sup>H]5-HT used in binding assays measures only the high affinity site of agonists? This is not likely, as it would not account for the data obtained with ICS-205-930 which is a silent antagonist in the present system displaying approximately 6 (CG-8) and 10 (CG-7) fold lower affinity in the functional assay as compared to the binding experiments. It is more likely that differences in experimental conditions used in binding assays compared with those used in the functional

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assays (such as membrane vs. cells, buffers and extent of equilibrium achieved) accounts for thes differences.

5        5-HT responses were resistant to blockade by methiothepin and spiperone (10  $\mu$ M). As the concentration of these agents exceed their equilibrium dissociation constants for their respective receptor sites by 10-100 fold, it seems that 5-HT<sub>1A</sub>,  
10      5-HT<sub>1B</sub>, 5-HT<sub>1C</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1E</sub>, 5-HT<sub>1F</sub>), 5-HT<sub>2</sub> and 5-HT<sub>4B</sub> receptors can be ruled out. In addition, the weak agonistic activity of 5-CT relative to 5-HT further supports the notion that 5-HT<sub>1</sub>-like receptors are probably not involved (Bradley, 1986). The results  
15      obtained with the indole agonists reflect those reported at the 5-HT<sub>4</sub> receptor in both the CNS and the periphery (Dumuis et al., 1988; Craig and Clarke, 1990; Eglen et al., 1990; Baxter, Craig and Clarke, 1991). The substituted benzamides, cisapride, BRL-24924 and zacopride acted as partial agonists. Although the benzamides also possess affinity for 5-HT<sub>3</sub> receptors, they lack intrinsic efficacy (Schuurkes et al., 1985; Sanger and King, 1988). Furthermore, the affinity of ICS-205-930 for antagonism of 5-HT response at S10 is  
20      1-3 orders of magnitude lower than that at 5-HT<sub>3</sub> receptors (Richardson et al., 1985) and therefore indicates a binding site different from 5-HT<sub>3</sub> receptor.  
25

As was the case with the rat 5-HT<sub>4</sub> receptor, there are two forms of the human homolog, most likely splice variants differing in the carboxyterminal end of the receptor. Non conservative amino acid substitutions, especially in transmembrane domains 1, 2 and 4 could provide the basis for the pharmacological differences observed between the rat and the human 5-HT<sub>4</sub> receptors (see below). A difference in the functional response is observed between the two rat c1 nes: although the

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CG-7 construct (S10-87) gives higher levels of receptor expression in COS-7 cells ( $B_{max}$  of 5.7 pmol/mg of protein versus 3.7 pmol/mg for S10-95), in the functional assay, the CG-8 construct (S10-95) 5 reproducibly shows a higher level of cAMP stimulation ( $2598 \pm 154\%$  of basal cAMP release versus  $2107 \pm 52\%$  for CG-7). This finding could be attributed to the different amino acid sequence in the carboxy terminal tail of the rat receptors, specially since the rat S10-10 95 isoform carries an additional potential phosphorylation site at position 400, absent in S10-87 (CG-7).

15 Since the human S10-95 homolog lacks the last 16 carboxy terminal amino acids which carry the phosphorylation site mentioned above in the rat homolog, it will be interesting to check for differences in the level of cAMP stimulation upon activation of the rat and human S10-95 homologs. In 20 the same way, after we get the full length human S10-87, both human isoforms will be compared in binding and functional assays.

25 All the unique pharmacological characteristics described above define the S10 genes as adenylate cyclase stimulatory "5-HT<sub>4</sub>" receptors reported in the literature that are expressed in the ileum (Craig and Clarke, 1990), hippocampus (Shenker et al., 1987), esophagus (Baxter et al., 1991), embryo colliculi 30 neurons (Dumius et al., 1988), atrium (Kaumann et al., 1990), adrenal (Lefebvre et al., 1992) and bladder (Corsi et al., 1991), and distinguish these clones from all other cloned subtypes of 5-HT receptor. Although 35 the binding profile of CG-7 and CG-8 were identical (Table 3), some differences in agonist potency (benzamides in particular) were observed between them in the functional assays. This is not surprising sinc

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the amino acid sequences of these two clones are identical, apart from the cytoplasmic carboxy tail, a region that is important for G protein-coupling, where the CG-8 receptor carries an extra phosphorylation site. Cisapride, BRL-24924 and zacopride had similar affinities at CG-8 whereas BRL-24924 and zacopride displayed approximately 4 and 15 fold lower affinity than cisapride at CG-7 clone for stimulation of adenylate cyclase. It is noteworthy that tissue differences in the potency of benzamides have been reported (Baxter et al., 1991) and whether this reflects a heterogeneity of 5-HT<sub>4</sub> receptors remains to be investigated.

The data obtained with the rat CG-7 and CG-8 clones and the human CG-17 clone using [<sup>3</sup>H]GR113808 are very similar to those reported by Grossmann et al. (1993) and Waeber et al. (1993) with this ligand using guinea pig and human brain tissues. Specific [<sup>3</sup>H]GR113808 binding readily saturated for all three clones (CG-7, CG-8 and CG-17). Scatchard analysis of specific binding in all three clones revealed the involvement of a single site. Curve fitting analysis showed an equilibrium dissociation of approximately 1 nM for both rat clones (CG-7 and CG-8) whereas this value was about 5-fold lower for the human CG-17 clone ( $K_d = 0.2$  nM). The  $K_d$  value obtained for the human CG-17 clone is in excellent agreement with that reported by Waeber et al. (1993) using human brain (0.23-0.37 nM) and is very similar to that of the guinea pig brain tissue (0.13-0.2 nM; Waeber et al., 1993; Grossmann et al., 1993). The  $K_d$  value of [<sup>3</sup>H]GR113808 for rat brain tissue has not been reported, however, it is interesting that the affinity obtained for GR113808 from functional receptor assays in the rat oesophagus (Grossmann et al., 1993) is about 0.3 nM which indicates that this antagonist has similar affinities for the human and the rat tissue

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used. The discrepancies between our data and those reported may be due to methodology, or different subtype (brain vs. oesophagus), however, this remains to be investigated.

5

The rank order of potency of compounds competing for specific [<sup>3</sup>H]GR113808 are very similar for both CG-7 and CG-8 and is cisapride > 5-HT > BRL-24924 > 5-MeOT = ICS205930 > zacopride >  $\alpha$ -Me-5-HT > 5-CT. This order of potency is different from that observed with guinea pig caudate (Grossmann et al., 1993; cisapride > 5-HT > ICS205930 > BRL-24924 > zacopride > 5-MeOT >  $\alpha$ -Me-5-HT > 5-CT) and human caudate (Waeber et al., 1993; cisapride > ICS205930 > BRL-24924 > 5-HT > 5-MeOT). Whether these differences are due to species or different population of high affinity state of the receptor in the various preparation, remains to be investigated. Interestingly, the displacement curves for most of the agonists competing for specific [<sup>3</sup>H]GR113808 were shallow and could be resolved to high and low affinity states. Gpp(NH)p shifted these curves to the right and in the case of 5-HT the Hill coefficient was increased to unity; however for some agonists the shift was not complete. Grossmann et al. (1993) using guinea pig caudate also observed shallow competition curves for some agonist that could be partially shifted by the addition of GTP (Grossmann et al., 1993). However, Waeber did not observe shallow binding curves with the human caudate tissue. These observations indicate that the G protein content of these preparations may be different which may reflect differences in the coupling of the receptor with a G protein.

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Using [<sup>3</sup>H]5-HT as the radioligand, the affinity values for the human CG-17 clone are in general comparable with that obtained previously for the rat CG-7 and CG-8 clones with few exceptions. The most striking

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5 differences appears to be for  $\alpha$ -Me-5-HT which displays approximat ly 100 fold higher affinity for the human CG-17. However, it has to pointed out that the data are compared with 2 different radioligands and this difference has to be further investigated using the same radioligand for all both the rat and human clones in parallel. 5-MeOT was approximately 3 fold less potent whereas zacopride was about 7 fold more potent at the human CG-17 as compared to the rat clones.

10 The cloning and expression of genes encoding 5-HT<sub>4</sub> receptors allows, for the first time, the ability to develop subtype selective drugs using radioligand binding assays. It will further provide definitive answers to whether there are significant species differences in the pharmacology of 5-HT<sub>4</sub> receptors.

15 In addition, the intrinsic activity of drugs can be determined from measures of adenylyl cyclase activation in these transfected cells. So far, native tissue preparations have shown great disparity in agonist activity. 5-HT<sub>4</sub> receptors have been implicated in a wide variety of functions. Existing drugs such as metaclopramide and cisapride appear to exert a large part of their action through 5-HT<sub>4</sub> receptors (Taniyama et al., 1991; Meulemans and Schurkes, 1992; Flynn et al., 1992). Experience with these agents indicates a clear therapeutic role for 5-HT<sub>4</sub> receptors in the gastrointestinal system for conditions including irritable bowel disease, postoperative ileus, diabetic gastroparesis, emesis, achalasia, hiatal hernia, and esophageal spasm. In addition, 5-HT<sub>4</sub> receptors have been described functionally in the heart (Kaumann, 1992), adrenal (Lefebvre et al., 1992), and bladder (Corsi et al., 1991) indicating possible roles in cardiac rate and force of contraction, endocrine control of cortisol secretion, and urinary incontinence or spasticity. 5-HT<sub>4</sub> recept rs have also been

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described in the brain, particularly in areas such as the hippocampus, in which we have localized the gene encoding 5-HT<sub>4</sub> receptors (S10-87), indicating a potential role in cognitive enhancement (Bockaert et al., 1992). As more specific pharmacological tools are developed, additional therapeutic indications will certainly be uncovered.

Table I: TH homology of the S10 receptor with other 7 TM receptors

TABLE 2

Primers	Cortex	Cerebellum	Brain Stem	Hippocampus	Olfactory Bulb	Striatum	Thalamus
	x	um	us	us	us	us	us
TM3-5	+	-	+	+	+	+	+
S10-87	ND	-	-	-	-	+	-
S10-95	ND	-	+	+	+	+	+

Table 2: PCR localization of the S10 mRNA in 7 different part of the rat brain.

The TM3-5 primers do not differentiate between clones S10-87 and S10-95. The S10-87 primers were designed from the nucleotide sequences coding for the TM 6 domain common to both receptors and for the carboxy terminus end specific to S10-87. In the same way, the S10-95 primers are specific for S10-95.

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**Table 3.** Binding affinities of key ligands for the identification of S10 (CG-7 and CG-8) as a 5-HT<sub>4</sub> receptor. Affinity constants (K<sub>i</sub>; nM) of drugs competing for S10 labeled with 10 nM [<sup>3</sup>H]5-HT were determined to pharmacologically define the encoded receptor as 5-HT<sub>4</sub>. K<sub>i</sub> (nM) values were calculated using the Cheng-Prusoff equation or estimated to be >1000 nM based upon one point displacements using a drug concentration of 1 uM. Affinity constants are expressed as the mean ± SEM (n≥2). K<sub>i</sub> values estimated to be >1000 were determined according to one point displacements studies at a concentration of 1 uM. (n=2 except BRL 24924 tested at CG-8: n=1)

#### CHARACTERIZATION OF CLONE B-10

##### Saturation Analysis:

$$K_d = 7.87 \pm 0.06 \text{ nM}$$

$$B_{max} = 1,938 \text{ fmol/mg prot}$$

##### Pharmacological profile:

DRUG	CG-7	CG-8
5-HT	8.6 ± 0.6	6.4 ± 0.5
Cisapride	10.9 ± 0.3	ND
5-MeOT	27.5 ± 5	ND
BRL 24924	27.7 ± 5	21.1*
ICS 205930	115 ± 12	138 ± 26
Zacopride	130 ± 10	136 ± 5
8OHDPAT	>1000	ND
Ketanserin	>1000	ND
Sumatriptan	>1000	ND
5-CT	>1000	ND

\* n = 1

ND = not determined

**Table 4.** Pharmacological profile for the cAMP response using the human 5-HT<sub>4A</sub> (CG-7 and CG-8) receptor transiently expressed in Cos-7 cells, comparison with the binding data obtained with CG-7 clone using [<sup>3</sup>H]5-HT. cAMP measurements on intact cells were as described under Methods and Materials. EC<sub>50</sub> values (concentration producing the half-maximal effect) were derived from the analysis of full dose-response curves. Maximum response produced by each drug was normalized to the 5-HT maximum response which is indicated as having an intrinsic activity of 1.0. Data are means ± S.E.M. of three separate experiments. The apparent dissociation constant of antagonist ( $K_d$ ) (IC<sub>50</sub> 205930) was calculated according to the formula:  $K_d = [B]/(A'/A) - 1$ , where [B] is the concentration of antagonist, A' and A the EC<sub>50</sub> values of agonist measured respectively in the presence and in the absence of antagonist (Furchtgott, 1972).

	EC <sub>50</sub> OR K <sub>d</sub> (nM)	I. A.	K <sub>i</sub> (nM)	K <sub>i</sub> (nM)
DRUG	CG-7	CG-8	CG-7	CG-8
5-MeOT	21 ± 6	31 ± 13	1.0	1.0
5-HT	26 ± 3	51 ± 7	1.0	1.0
Cisapride	191 ± 26	413 ± 199	1.2	1.4
α-Me-5-HT	250 ± 91	1,038 ± 31	0.90	1.0
BRL-24924	736 ± 129	250 ± 25	1.1	0.9
Zacopride	2,740 ± 274	239 ± 33	1.1	1.0
5-CT	5,570 ± 808	1,411 ± 211	0.85	1.2
ICS-205930	962 ± 244	607 ± 30	0	0

ND, not determined.

Maximum response to 5-HT in Cos-7 cells transiently transfected with CG-7 and CG-8 genes was:

CG-7= 2,107 ± 52 % of basal cAMP release

CG-8= 2,598 ± 154 % of basal cAMP release

Cisapride, BRL-24924 and zacopride had no antagonist activity and ICS-205930 had no intrinsic agonist activity.

**Table 5.** The affinities of various compounds that compete for 0.2-0.4 nM [<sup>3</sup>H]GR113808 binding in membranes of COS-7 cells transiently transfected with rat clones CG-7 and CG-8.

COMPOUNDS	CG-7	CG-8
5-HT	237 $K_h=2.6$ , $BH=22\pm$ $K_L=357$ $nH=0.62$	116 $KH=2.5$ , $BH=24\pm$ $KL=197$ $nH=0.67$
5-CT	>10,000	>10,000
5-Me-OT	438 $KH=14$ , $BH=17\pm$ $KL=658$ $nH=0.57$	518 $KH=$ , $BH=$ $KL=$ $nH=0.66$
BRL-24924	189 $KH=37$ , $BH=34\pm$ $KL=371$ $nH=0.85$	188 $KH=23$ , $BH=32\pm$ $KL=373$ $nH=0.81$
ZACOPRIDE	729 $KH=424$ , $BH=68\pm$ $KL=2,757$ $nH=0.80$	820 $KH=$ , $BH=$ $KL=$ $nH=0.85$
d-LSD	>10,000	>10,000
CISAPRIDE	ND	84 $KH=2.7$ , $BH=11\pm$ $KL=105$ $nH=0.83$
ICS205930	ND	529
$\alpha$ -Me-5-HT	2,255	1,855

Affinity estimates are given as  $K_i$  values (nM) using the computer program "Accucomp" (Lundon Software). Values are from a single experiment.  $K_h$  and  $K_L$  values indicate the affinity in nM for the high affinity and low affinity state of the receptor,  $B_h$  is the percentage of high affinity sites and  $nH$  is the Hill coefficient.  
ND, not determined.

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**Table 6. Binding affinities of key ligands for the identification of the human CG-17 clone as a 5-HT<sub>4A</sub> receptor.**

COMPOUND	K <sub>i</sub> (nM)
5-HT	4.2
5-MeOT	71
5-CT	>10,000
Cisapride	12
$\alpha$ -Me-5-HT	1.6
BRL-24924	21
Zacopride	17
Sumatriptan	>1,000
8-OH-DPAT	>1,000
Ketanserin	>1,000

Affinity constants (K<sub>i</sub>, nM) of drugs competing for CG-17 cloned labeled with 10 nM [<sup>3</sup>H]5-HT were calculated using the Cheng-Prusoff equation or estimated to be greater than 1,000 nM based upon one point displacement using a drug concentration of 1  $\mu$ M. Values are from a single experiment.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: SYNAPTIC PHARMACEUTICAL CORPORATION

(ii) TITLE OF INVENTION: DNA ENCODING 5-HT<sub>4</sub> SEROTONIN RECEPTORS  
AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: COOPER & DUNHAM
- (B) STREET: 30 ROCKEFELLER PLAZA
- (C) CITY: NEW YORK
- (D) STATE: NEW YORK
- (F) ZIP: 10112

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: Patentin Release #1.24

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: White, P., John
- (B) REGISTRATION NUMBER: 28,678
- (C) REFERENCE/DOCKET NUMBER: 42667-A-PCT/JPW/TEP

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (212) 977-9550
- (B) TELEFAX: (212) 664-0525
- (C) TELEX: 422523 COOP UI

## (2) INFORMATION FOR SEQ ID NO:1:

-83-

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1642 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:

- (F) TISSUE TYPE: brain

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: rat brain
- (B) CLONE: S10-87

(ix) FEATURE:

- (A) NAME/KEY: cDS
- (B) LOCATION: 101..1261
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGCCTTGGCG	AGCCTGGCTT	GGTTGGAAGG	AGGAGGATGC	TCTGCCGTGCC	CAGGGTCCCTG	60
TGGGCACTGA	CATCCAACGT	ACTCATGCC	ATTCCTGTAA	ATG GAC AGA CTT GAT		115
				Met Asp Arg Leu Asp		
				1	5	
GCT AAT GTG ACT TCC AAC GAG GGT TTC GGG TCT GTG GAG AAG GTC GTA						163
Ala Asn Val Ser Ser Asn Glu Gly Phe Gly Ser Val Glu Lys Val Val						
10			15		20	
CTG CTC ACG TTC TTC GCA ATG GTT ATC CTG ATG GCC ATC CTG GCC AAC						211
Leu Leu Thr Phe Phe Ala Met Val Ile Leu Met Ala Ile Leu Gly Asn						
25			30		35	
CTG CTG GTG ATG GTT GCT GTG TGC AGG GAC AGG CAG CTC AGG AAA ATA						259
Leu Leu Val Met Val Ala Val Cys Arg Asp Arg Gln Leu Arg Lys Ile						
40		45		50		
AAA ACC AAT TAT TTC ATT GTG TCT CTT GCC TTT GCT GAT CTG CTG GTT						307

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Lys	Thr	Asn	Tyr	Phe	Ile	Val	Ser	Leu	Ala	Phe	Ala	Asp	Leu	Leu	Val	
55					60						65					
TCG	GTG	CTG	GTG	AAT	GCC	TTC	GGT	GCC	ATT	GAG	TTG	GTC	CAA	GAC	ATC	355
Ser	Val	Leu	Val	Asn	Ala	Phe	Gly	Ala	Ile	Glu	Leu	Val	Gln	Asp	Ile	
70					75					80						85
TGG	TTT	TAT	GGG	GAG	ATG	TTT	TGC	CTG	GTC	CGG	ACC	TCT	CTG	GAT	GTC	403
Trp	Phe	Tyr	Gly	Glu	Met	Phe	Cys	Leu	Val	Arg	Thr	Ser	Leu	Asp	Val	
					90					95					100	
CTA	CTC	ACC	ACA	GCA	TCA	ATT	TTT	CAC	CTC	TGC	TGC	ATT	TCC	CTG	GAT	451
Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His	Leu	Cys	Cys	Ile	Ser	Leu	Asp	
					105				110						115	
AGG	TAT	TAT	GCC	ATC	TGC	TGT	CAA	CCT	TTG	GTT	TAT	AGA	AAC	AAG	ATG	499
Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro	Leu	Val	Tyr	Arg	Asn	Lys	Met	
					120				125						130	
ACC	CCT	CTA	CGC	ATC	GCA	TTA	ATG	CTG	GGA	GSC	TGC	TGG	GTC	ATT	CCC	547
Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu	Gly	Gly	Cys	Trp	Val	Ile	Pro	
					135				140						145	
ATG	TTT	ATA	TCT	TTT	CTC	CCC	ATA	ATG	CAA	GCC	TGG	AAC	AAC	ATC	GGC	595
Met	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met	Gln	Gly	Trp	Asn	Asn	Ile	Gly	
					150				155						160	
ATA	GTT	GAT	GTG	ATA	GAG	AAA	AGG	AAA	TTC	AAC	CAC	AAC	TCT	AAC	TCT	643
Ile	Val	Asp	Val	Ile	Glu	Lys	Arg	Lys	Phe	Asn	His	Asn	Ser	Asn	Ser	
					170				175						180	
ACA	TTC	TGT	GTC	TTC	ATG	GTC	AAC	AAG	CCC	TAT	GCC	ATC	ACC	TGC	TCT	691
Thr	Phe	Cys	Val	Phe	Met	Val	Asn	Lys	Pro	Tyr	Ala	Ile	Thr	Cys	Ser	
					185				190						195	
GTG	GTG	GCC	TTC	TAC	ATC	CCG	TTT	CTC	CTC	ATG	GTG	CTG	GCC	TAT	TAC	739
Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu	Leu	Met	Val	Leu	Ala	Tyr	Tyr	
					200				205						210	
CGT	ATC	TAT	GTC	ACT	GCT	AAG	GAG	CAT	GCC	CAG	CAG	ATC	CAG	ATG	TTA	787
Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His	Ala	Gln	Gln	Ile	Gln	Met	Leu	
					215				220						225	
CAA	CGG	GCA	GGA	GCC	ACC	TCT	GAA	AGC	AGG	CCC	CAG	ACA	GCT	GAC	CAG	835
Gln	Arg	Ala	Gly	Ala	Thr	Ser	Glu	Ser	Arg	Pro	Gln	Thr	Ala	Asp	Gln	

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230	235	240	245	883
CAC AGC ACA CAT CGC ATG CGG ACA GAG ACC AAA GCA GCC AAG ACT TTA				
His Ser Thr His Arg Met Arg Thr Glu Thr Lys Ala Ala Lys Thr Leu				
250	255	260		
TGT GTC ATC ATG GCC TGC TTC TGT TTC TGC TGG GCC CCC TTC TTT GTC				931
Cys Val Ile Met Gly Cys Phe Cys Phe Cys Trp Ala Pro Phe Phe Val				
265	270	275		
ACC AAT ATT GTG GAC CCT TTC ATA GAC TAC ACT GTG CCC GAG AAG GTG				979
Thr Asn Ile Val Asp Pro Phe Ile Asp Tyr Thr Val Pro Glu Lys Val				
280	285	290		
TGG ACT GCT TTC CTC TGG CTT GCC TAT ATC AAT TCA GGG TTG AAC CCT				1027
Trp Thr Ala Phe Leu Trp Leu Gly Tyr Ile Asn Ser Gly Leu Asn Pro				
295	300	305		
TTT CTC TAT GCC TTC TTG AAT AAG TCT TTC AGA CGT GCC TTC CTT ATC				1075
Phe Leu Tyr Ala Phe Leu Asn Lys Ser Phe Arg Arg Ala Phe Leu Ile				
310	315	320	325	
ATC CTC TGC TGT GAT GAT GAG CGC TAC AAA AGA CCC CCC ATT CTG GGC				1123
Ile Leu Cys Cys Asp Asp Glu Arg Tyr Lys Arg Pro Pro Ile Leu GLY				
330	335	340		
CAG ACT GTC CCC TGT TCA ACC ACA ACC ATT AAT GGA TCC ACT CAT GTG				1171
Gln Thr Val Pro Cys Ser Thr Thr Ile Asn Gly Ser Thr His Val				
345	350	355		
CTA AGG TAT ACA GTT TTG CAT AGT GGT CAA CAC CAG GAA CTG GAG AAC				1219
Leu Arg Tyr Thr Val Leu His Ser Gly Gln His Gln Glu Leu Glu Lys				
360	365	370		
TTG CCC ATA CAC AAT GAC CCA GAG TCC CTG GAA TCA TGC TTT				1261
Leu Pro Ile His Asn Asp Pro Glu Ser Leu Glu Ser Cys Phe				
375	380	385		
TGATTGAAGA CGTGGCTTGC CTTTAGCGGT TCATCCCATC TGTGTCTGCA TGAACAGGTT				1321
ACTATGGAAT CACTCCTGAC TCTGGGCATC ACCAGTGAAAC CATGAGCATG GTGAGGCAGG				1381
GTCCCCGTGAA GGTGCCACAGA GGACAGCATT GAGTGGGACC TGAACCCAGC ACATTAAGGA				1461
TTTCAGAACCG CGTGTGGGAT TTGAGATGTC ATCAGACCCA GTGTCTTACCG CAGAGCCCAA				1501

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CTGGCACCTC	CCATCCACG	CTGACATGTG	GTCAGTCTTT	GCTCACACCT	CTCCAGGCC	1561
AGGAGCTGAC	TACCTCTAA	TGTGGTGGG	AGCTCTTAAT	TGTGTGGAAG	TTCAGTCATT	1621
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## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 387 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Asp	Arg	Leu	Asp	Ala	Asn	Val	Ser	Ser	Asn	Glu	Gly	Phe	Gly	Ser
1				5					10					15	
Val	Glu	Lys	Val	Val	Leu	Leu	Thr	Phe	Phe	Ala	Met	Val	Ile	Leu	Met
				20				25					30		
Ala	Ile	Leu	Gly	Asn	Leu	Leu	Val	Met	Val	Ala	Val	Cys	Arg	Asp	Arg
				35			40				45				
Gln	Leu	Arg	Lys	Ile	Lys	Thr	Asn	Tyr	Phe	Ile	Val	Ser	Leu	Ala	Phe
					55				60						
Ala	Asp	Leu	Leu	Val	Ser	Val	Leu	Val	Asn	Ala	Phe	Gly	Ala	Ile	Glu
				65		70			75					80	
Leu	Val	Gln	Asp	Ile	Trp	Phe	Tyr	Gly	Glu	Met	Phe	Cys	Leu	Val	Arg
					85				90				95		
Thr	Ser	Leu	Asp	Val	Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His	Leu	Cys
				100			105				110				
Cys	Ile	Ser	Leu	Asp	Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro	Leu	Val
				115			120				125				
Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu	Gly	Gly
				130			135				140				

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Cys	Trp	Val	Ile	Pro	Met	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met	Gln	Gly
145					150					155					160
Trp	Asn	Asn	Ile	Gly	Ile	Val	Asp	Val	Ile	Glu	Lys	Arg	Lys	Phe	Asn
					165					170					175
His	Asn	Ser	Asn	Ser	Thr	Phe	Cys	Val	Phe	Met	Val	Asn	Lys	Pro	Tyr
					180					185					190
Ala	Ile	Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu	Leu	Met
					195					200					205
Val	Leu	Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His	Ala	Gln
					210			215				220			
Gln	Ile	Gln	Met	Leu	Gln	Arg	Ala	Gly	Ala	Thr	Ser	Glu	Ser	Arg	Pro
					225			230			235				240
Gln	Thr	Ala	Asp	Gln	His	Ser	Thr	His	Arg	Met	Arg	Thr	Glu	Thr	Lys
					245				250						255
Ala	Ala	Lys	Thr	Leu	Cys	Val	Ile	Met	Gly	Cys	Phe	Cys	Phe	Cys	Trp
					260			265							270
Ala	Pro	Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp	Tyr	Thr
					275			280							285
Val	Pro	Glu	Lys	Val	Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr	Ile	Asn
					290			295				300			
Ser	Gly	Leu	Asn	Pro	Phe	Leu	Tyr	Ala	Phe	Leu	Asn	Lys	Ser	Phe	Arg
					305			310			315				320
Arg	Ala	Phe	Leu	Ile	Ile	Leu	Cys	Cys	Asp	Asp	Glu	Arg	Tyr	Lys	Arg
					325				330						335
Pro	Pro	Ile	Leu	Gly	Gln	Thr	Val	Pro	Cys	Ser	Thr	Thr	Thr	Ile	Asn
					340			345							350
Gly	Ser	Thr	His	Val	Leu	Arg	Tyr	Thr	Val	Leu	His	Ser	Gly	Gln	His
					355			360				365			
Gln	Glu	Leu	Glu	Lys	Leu	Pro	Ile	His	Asn	Asp	Pro	Glu	Ser	Leu	Glu
					370			375			380				
Ser	Cys	Phe													

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## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1622 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:

- (F) TISSUE TYPE: brain

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: rat brain
- (B) CLONE: S10-95

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 50..1267
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGGGTCTGT	GGGCACTGAC	ATCCAACGTA	CTCATGCCCA	TTTCCTGTA	ATG	GAC	55									
					Met	Asp										
							1									
AGA	CTT	GAT	GCT	AAT	GTG	AGT	TCC	AAC	GAG	GGT	TTC	GGG	TCT	GTG	GAG	103
Arg	Leu	Asp	Ala	Asn	Val	Ser	Ser	Asn	Glu	Gly	Phe	Gly	Ser	Val	Glu	
5					10								15			
AAG	GTC	GTA	CTG	CTC	ACG	TTC	TTC	GCA	ATG	GTT	ATC	CTG	ATG	GCC	ATC	151
Lys	Val	Val	Leu	Leu	Thr	Phe	Phe	Ala	Met	Val	Ile	Leu	Met	Ala	Ile	
20					25								30			
CTG	GGC	AAC	CTG	CTG	GTG	ATG	GTT	GCT	GTG	TGC	AGG	GAC	AGG	CAG	CTC	199
Leu	Gly	Asn	Leu	Leu	Val	Met	Val	Ala	Val	Cys	Arg	Asp	Arg	Gln	Leu	

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35	40	45	50	
AGG AAA ATA AAA ACC AAT TAT TTC ATT GTG TCT CTT GCC TTT GCT GAT				247
Arg Lys Ile Lys Thr Asn Tyr Phe Ile Val Ser Leu Ala Phe Ala Asp				
55	60		65	
CTG CTG GTT TCG GTG CTG GTG AAT GCC TTC GGT GCC ATT GAG TTG GTC				295
Leu Leu Val Ser Val Leu Val Asn Ala Phe Gly Ala Ile Glu Leu Val				
70	75		80	
CAA GAC ATC TGG TTT TAT GGG GAG ATG TTT TGC CTG GTC CGG ACC TCT				343
Gln Asp Ile Trp Phe Tyr Gly Glu Met Phe Cys Leu Val Arg Thr Ser				
85	90		95	
CTG GAT GTC CTA CTC ACC ACA GCA TCA ATT TTT CAC CTC TCC TGC CTT				391
Leu Asp Val Leu Leu Thr Thr Ala Ser Ile Phe His Leu Cys Cys Leu				
100	105		110	
TCC CTG GAT AGG TAT TAT GCC ATC TCC TGT CAA CCT TTG GTT TAT AGA				439
Ser Leu Asp Arg Tyr Tyr Ala Ile Cys Cys Gln Pro Leu Val Tyr Arg				
115	120		125	
AAC AAG ATG ACC CCT CTA CGC ATC GCA TTA ATG CTG GGA GGC TGC TGG				487
Asn Lys Met Thr Pro Leu Arg Ile Ala Leu Met Leu Gly Gly Cys Trp				
135	140		145	
GTC ATT CCC ATG TTT ATA TCT TTT CTC CCC ATA ATG CAA GGC TGG AAC				535
Val Ile Pro Met Phe Ile Ser Phe Leu Pro Ile Met Gln Gly Trp Asn				
150	155		160	
AAC ATC GGC ATA GTT GAT GTG ATA GAG AAA AGG AAA TTC AAC CAC AAC				583
Asn Ile Gly Ile Val Asp Val Ile Glu Lys Arg Lys Phe Asn His Asn				
165	170		175	
TCT AAC TCT ACA TTC TGT GTC TTC ATG GTC AAC AAG CCC TAT GCC ATC				631
Ser Asn Ser Thr Phe Cys Val Phe Met Val Asn Lys Pro Tyr Ala Ile				
180	185		190	
ACC TGC TCT GTG GTG GCC TTC TAC ATC CCG TTT CTC CTC ATG GTG CTG				679
Thr Cys Ser Val Val Ala Phe Tyr Ile Pro Phe Leu Leu Met Val Leu				
195	200		205	
GCC TAT TAC CGT ATC TAT GTC ACT GCT AAG GAG CAT GCC CAG CAG ATC				727
Ala Tyr Tyr Arg Ile Tyr Val Thr Ala Lys Glu His Ala Gln Gln Ile				
215	220		225	

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CAG	ATG	TTA	CAA	CCG	GCA	GGA	GCC	ACC	TCT	GAA	AGC	AGG	CCC	CAG	ACA		775
Gln	Met	Ieu	Gln	Arg	Ala	Gly	Ala	Thr	Ser	Glu	Ser	Arg	Pro	Gln	Thr		
			230					235					260				
GCT	GAC	CAG	CAC	AGC	ACA	CAT	CCC	ATG	CGG	ACA	GAG	ACC	AAA	GCA	GCC		823
Ala	Asp	Gln	His	Ser	Thr	His	Arg	Met	Arg	Thr	Glu	Thr	Lys	Ala	Ala		
			245				250					255					
AAG	ACT	TTA	TGT	GTC	ATC	ATG	GGC	TGC	TTC	TGT	TTC	TCC	TGG	GCC	CCC		871
Lys	Thr	Ieu	Cys	Val	Ile	Met	Gly	Cys	Phe	Cys	Phe	Cys	Trp	Ala	Pro		
			260			265			270								
TTC	TTT	GTC	ACC	AAT	ATT	GTG	GAC	CCT	TTC	ATA	GAC	TAC	ACT	GTG	CCC		919
Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp	Tyr	Thr	Val	Pro		
			275			280			285					290			
GAG	AAG	GTG	TGG	ACT	GCT	TTC	CTC	TGG	CTT	GGC	TAT	ATC	AAT	TCA	GGG		967
Glu	Lys	Val	Trp	Thr	Ala	Phe	Ieu	Trp	Leu	Gly	Tyr	Ile	Asn	Ser	Gly		
			295				300						305				
TTG	AAC	CCT	TTT	CTC	TAT	GCC	TTC	TTG	AAT	AAG	TCT	TTC	AGA	CGT	GCC		1015
Leu	Asn	Pro	Phe	Leu	Tyr	Ala	Phe	Leu	Asn	Lys	Ser	Phe	Arg	Arg	Ala		
			310				315					320					
TTC	CTT	ATC	ATC	CTC	TGC	TGT	GAT	GAT	GAG	GGC	TAC	AAA	AGA	CCC	CCC		1063
Phe	Ieu	Ile	Ile	Leu	Cys	Cys	Asp	Asp	Glu	Arg	Tyr	Lys	Arg	Pro	Pro		
			325			330						335					
ATT	CTG	GGC	CAG	ACT	GTC	CCC	TGT	TCA	ACC	ACA	ACC	ATT	AAT	GGA	TCC		1111
Ile	Ieu	Gly	Gln	Thr	Val	Pro	Cys	Ser	Thr	Thr	Thr	Ile	Asn	Gly	Ser		
			340			345			350								
ACT	CAT	GTG	CTA	AGG	GAT	ACA	GTG	GAA	TCT	GGT	GGC	CAA	TGG	GAG	ACT		1159
Thr	His	Val	Leu	Arg	Asp	Thr	Val	Glu	Cys	Gly	Gly	Gln	Trp	Glu	Ser		
			355			360			365				370				
CGG	TGT	CAC	CTC	ACA	GCA	ACT	TCT	CCT	TTG	GTG	GCT	GCT	CAG	CCA	GTG		1207
Arg	Cys	His	Leu	Thr	Ala	Thr	Ser	Pro	Leu	Val	Ala	Ala	Gln	Pro	Val		
			375				380						385				
ATA	CGT	AGG	CCC	CAG	GAC	AAT	GAC	CTA	GAA	GAC	AGC	TGT	AGC	TTG	AAA		1255
Ile	Arg	Arg	Pro	Gln	Asp	Asn	Asp	Leu	Glu	Asp	Ser	Cys	Ser	Leu	Lys		
			390			395			400								
AGA	AGC	CAG	TCC	TAAGCTGCTA		CTTCGGTGTA		TGTGGCTGCC		CCTGGCACTT						1307	

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Arg Ser Gln Ser  
405

TGTTTCTCCAA	GGCTTTCCAA	GACCATGAGG	CAATCCACCC	TGGACTTCCC	GCCACGGATTC	1367
TAGCAGGGGG	TATTAGAGGA	AGTCAGGGGA	GAGAAGGCCT	TCTCTCTTAG	CTTCTGTGTT	1427
CTCAACATTT	TCTCTTCCTG	GAGTCCTCAC	TCTTGCTTGG	TGGTCTCTGA	AGTCCACGAC	1487
CCAGTCCCCCT	TTTGTGTCT	CCAGTCTGTC	TTGTAAATCT	TTACCGTGT	CGATTTCAAG	1547
TTTCCAAACA	TGCCCTCTTT	GAAGTGTCA	CTTACGATA	TGTAAAACA	TGTGCCTGTC	1607
TTGATCACAC	TTCTT					1622

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 406 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Asp	Arg	Leu	Asp	Ala	Asn	Val	Ser	Ser	Asn	Glu	Gly	Phe	Gly	Ser
1				5					10					15	
Val	Glu	Lys	Val	Val	Leu	Leu	Thr	Phe	Phe	Ala	Met	Val	Ile	Leu	Met
				20					25					30	
Ala	Ile	Leu	Gly	Asn	Leu	Leu	Val	Met	Val	Ala	Val	Cys	Arg	Asp	Arg
				35			40					45			
Gln	Leu	Arg	Lys	Ile	Lys	Thr	Asn	Tyr	Phe	Ile	Val	Ser	Leu	Ala	Phe
						55					60				
Ala	Asp	Leu	Leu	Val	Ser	Val	Leu	Val	Asn	Ala	Phe	Gly	Ala	Ile	Glu
				65		70				75				80	
Leu	Val	Gln	Asp	Ile	Trp	Phe	Tyr	Gly	Glu	Met	Phe	Cys	Leu	Val	Arg
									90					95	

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Thr	Ser	Leu	Asp	Val	Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His	Leu	Cys
				100				105					110		
Cys	Leu	Sr	Leu	Asp	Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro	Leu	Val
						115		120				125			
Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu	Gly	Gly
					130		135				140				
Cys	Trp	Val	Ile	Pro	Met	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met	Gln	Gly
					145	150			155				160		
Trp	Asn	Asn	Ile	Gly	Ile	Val	Asp	Val	Ile	Glu	Lys	Arg	Lys	Phe	Asn
					165				170			175			
His	Asn	Ser	Asn	Ser	Thr	Phe	Cys	Val	Phe	Met	Val	Asn	Lys	Pro	Tyr
					180			185				190			
Ala	Ile	Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu	Leu	Met
					195		200				205				
Val	Leu	Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His	Ala	Gln
					210		215				220				
Gln	Ile	Gln	Met	Leu	Gln	Arg	Ala	Gly	Ala	Thr	Ser	Glu	Ser	Arg	Pro
					225	230				235			240		
Gln	Thr	Ala	Asp	Gln	His	Ser	Thr	His	Arg	Met	Arg	Thr	Glu	Thr	Lys
					245			250				255			
Ala	Ala	Lys	Thr	Leu	Cys	Val	Ile	Met	Gly	Cys	Phe	Cys	Phe	Cys	Trp
					260		265				270				
Ala	Pro	Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp	Tyr	Thr
					275		280				285				
Val	Pro	Glu	Lys	Val	Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr	Ile	Asn
					290		295				300				
Ser	Gly	Leu	Asn	Pro	Phe	Leu	Tyr	Ala	Phe	Leu	Asn	Lys	Ser	Phe	Arg
					305		310				315			320	
Arg	Ala	Ph	Leu	Ile	Ile	Leu	Cys	Cys	Asp	Asp	Glu	Arg	Tyr	Lys	Arg
					325				330			335			

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Pro	Pro	Ile	Leu	Gly	Gln	Thr	Val	Pro	Cys	Ser	Thr	Thr	Thr	Ile	Asn
			340					345						350	
Gly	Ser	Thr	His	Val	Leu	Arg	Asp	Thr	Val	Glu	Cys	Gly	Gly	Gln	Trp
			355					360				365			
Glu	Ser	Arg	Cys	His	Leu	Thr	Ala	Thr	Ser	Pro	Leu	Val	Ala	Ala	Gln
			370			375					380				
Pro	Val	Ile	Arg	Arg	Pro	Gln	Asp	Asn	Asp	Leu	Glu	Asp	Ser	Cys	Ser
	385					390				395					400
Leu	Lys	Arg	Ser	Gln	Ser										
						405									

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 536 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(v) ORIGINAL SOURCE:

(F) TISSUE TYPE: brain

(vi) IMMEDIATE SOURCE:

- (A) LIBRARY: human brain
- (B) CLONE: S10 PCR

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..534
- (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTG GTC TAT AGG AAC AAG ATG ACC CCT CTG CGC ATC GCA TTA ATG CTG

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Lew	Val	Tyr	Arg	Asn	Lys	Met	Thr	Pro	Lew	Arg	Ile	Ala	Lew	Met	Lew	
1				5					10					15		
GGA	GCC	TGC	TGG	GTC	ATC	CCC	ACG	TTT	ATT	TCT	TTT	CTC	CCT	ATA	ATG	96
Gly	Gly	Cys	Trp	Val	Ile	Pro	Thr	Phe	Ile	Ser	Phe	Lew	Pro	Ile	Met	
				20					25					30		
CAA	GGC	TGG	AAT	AAC	ATT	GGC	ATA	ATT	GAT	TTG	ATA	GAA	AAG	AGG	AAG	164
Gln	Gly	Trp	Asn	Asn	Ile	Gly	Ile	Ile	Asp	Leu	Ile	Glu	Lys	Arg	Lys	
				35				40				45				
TTC	AAC	CAG	AAC	TCT	AAC	TCT	ACG	TAC	TGT	GTC	TTC	ATG	GTC	AAC	AAG	192
Phe	Asn	Gln	Asn	Ser	Asn	Ser	Thr	Tyr	Cys	Val	Phe	Met	Val	Asn	Lys	
				50			55				60					
CCC	TAC	GCC	ATC	ACC	TGC	TCT	GTG	GTG	GCC	TTC	TAC	ATC	CCA	TTT	CTC	240
Pro	Tyr	Ala	Ile	Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Lew	
				65			70			75				80		
CTC	ATG	GTC	CTG	GCC	TAT	TAC	CGC	ATC	TAT	GTC	ACA	GCT	AAG	GAG	CAT	288
Lew	Met	Val	Lew	Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His	
				85			90				95					
GCC	CAT	CAG	ATC	CAG	ATG	TTA	CAA	CGG	GCA	GGA	GCC	TCC	TCC	GAG	AGC	336
Ala	His	Gln	Ile	Gln	Met	Lew	Gln	Arg	Ala	Gly	Ala	Ser	Ser	Glu	Ser	
				100			105				110					
AGG	CCT	CAG	TCG	GCA	GAC	CAG	CAT	AGC	ACT	CAT	CCG	ATG	AGG	ACA	GAG	384
Arg	Pro	Gln	Ser	Ala	Asp	Gln	His	Ser	Thr	His	Pro	Met	Arg	Thr	Glu	
				115			120			125						
ACC	AAA	GCA	GCC	AAG	ACC	CTG	TGC	ATC	ATC	ATG	GGT	TGC	TTC	TGC	CTC	432
Thr	Lys	Ala	Ala	Lys	Thr	Lew	Cys	Ile	Ile	Met	Gly	Cys	Phe	Cys	Lew	
				130			135				140					
TGC	TGG	GCA	CCA	TTC	TTT	GTC	ACC	AAT	ATT	GTG	GAT	CCT	TTC	ATA	GAC	480
Cys	Trp	Ala	Pro	Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp	
				145			150			155				160		
TAC	ACT	GTC	CCT	GGG	CAG	GTG	TGG	ACT	GCT	TTC	CTC	TGG	CTC	GGC	TAT	528
Tyr	Thr	Val	Pro	Gly	Gln	Val	Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr	
				165			170				175					
ATC	AAT	TC														536
Ile	Asn															

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## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 178 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu	Val	Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu
1									10						15
Gly	Gly	Cys	Trp	Val	Ile	Pro	Thr	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met
				20					25						30
Gln	Gly	Trp	Asn	Asn	Ile	Gly	Ile	Ile	Asp	Leu	Ile	Glu	Lys	Arg	Lys
								35				45			
Phe	Asn	Gln	Asn	Ser	Asn	Ser	Thr	Tyr	Cys	Val	Phe	Met	Val	Asn	Lys
								50				60			
Pro	Tyr	Ala	Ile	Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu
								65				75			80
Leu	Met	Val	Leu	Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His
									85						95
Ala	His	Gln	Ile	Gln	Met	Leu	Gln	Arg	Ala	Gly	Ala	Ser	Ser	Glu	Ser
								100				110			
Arg	Pro	Gln	Ser	Ala	Asp	Gln	His	Ser	Thr	His	Pro	Met	Arg	Thr	Glu
								115				125			
Thr	Lys	Ala	Ala	Lys	Thr	Leu	Cys	Ile	Ile	Met	Gly	Cys	Phe	Cys	Leu
								130			140				
Cys	Trp	Ala	Pro	Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp
									145						160
Tyr	Thr	Val	Pro	Gly	Gln	Val	Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr
									165			170			175

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Ile Asn

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1316 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:

- (F) TISSUE TYPE: Brain

(vi) IMMEDIATE SOURCE:

- (A) LIBRARY: Human Brain
- (B) CLONE: S10-87

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 7..1170

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCTGTA	ATG	GAC	AAA	CTT	GAT	GCT	AAT	GTG	ACT	TCT	GAG	GAG	GGT	TTC		48
	Met	Asp	Lys	Leu	Asp	Ala	Asn	Val	Ser	Ser	Glu	Glu	Gly	Phe		
	1				5					10						
GGG	TCA	GTC	GAG	AAG	GTG	GTG	CTG	CTC	ACG	TTT	CTC	TCG	ACG	GTT	ATC	96
Gly	Ser	Val	Glu	Lys	Val	Val	Leu	Leu	Thr	Phe	Leu	Ser	Thr	Val	Ile	
	15				20					25				30		
CTG	ATG	GCC	ATC	TTG	GGG	AAC	CTG	CTG	GTG	ATG	GTG	GCT	GTG	TGC	TGG	144
Leu	Met	Ala	Ile	Leu	Gly	Asn	Leu	Leu	Val	Met	Val	Ala	Val	Cys	Trp	
	35							40						45		
GAC	AGG	CAG	CTC	AGG	AAA	ATA	AAA	ACA	AAT	TAT	TTC	ATT	GTA	TCT	CTT	192
Asp	Arg	Gln	Leu	Arg	Lys	Ile	Lys	Thr	Asn	Tyr	Phe	Ile	Val	Ser	Leu	
	50							55				60				

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GCT	TTT	GCG	GAT	CTG	CTG	GTT	TGG	GTC	CTG	GTC	ATG	CCC	TTT	GGT	GCC		260
Ala	Phe	Ala	Asp	Leu	Leu	Val	Ser	Val	Leu	Val	Met	Pro	Phe	Gly	Ala		
65						70						75					
ATT	GAG	CTG	GTT	CAA	GAC	ATC	TGG	ATT	TAT	GGG	GAG	GTC	TTT	TGT	CTT		288
Ile	Glu	Leu	Val	Gln	Asp	Ile	Trp	Ile	Tyr	Gly	Glu	Val	Phe	Cys	Leu		
80						85				90							
GTT	CGG	ACA	TCT	CTG	GAC	GTC	CTG	CTC	ACA	ACG	GCA	TCG	ATT	TTT	CAC		336
Val	Arg	Thr	Ser	Leu	Asp	Val	Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His		
95					100				105						110		
CTG	TGC	TGC	ATT	TCT	CTG	GAT	AGG	TAT	TAC	GCC	ATC	TGC	TGC	CAG	CCT		384
Leu	Cys	Cys	Ile	Ser	Leu	Asp	Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro		
					115				120						125		
TTG	GTC	TAT	AGG	AAC	AAG	ATG	ACC	CCT	CTG	GGC	ATC	GCA	TTA	ATG	CTG		432
Leu	Val	Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu		
					130				135						140		
GGA	GGC	TGC	TGG	GTC	ATC	CCC	ACG	TTT	ATT	TCT	TTT	CTC	CCT	ATA	ATG		480
Gly	Gly	Cys	Trp	Val	Ile	Pro	Thr	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met		
					145			150							155		
CAA	GCC	TGG	AAT	AAC	ATT	GGC	ATA	ATT	GAT	TTG	ATA	GAA	AAG	AGG	AAG		528
Gln	Gly	Trp	Asn	Asn	Ile	Gly	Ile	Ile	Asp	Leu	Ile	Glu	Lys	Arg	Lys		
					160			165				170					
TTC	AAC	CAG	AAC	TCT	AAC	TCT	ACG	TAC	TGT	GTC	TTC	ATG	GTC	AAC	AAG		576
Phe	Asn	Gln	Asn	Ser	Asn	Ser	Thr	Tyr	Cys	Val	Phe	Met	Val	Asn	Lys		
					175			180			185				190		
CCC	TAC	GCC	ATC	ACC	TGC	TCT	GTC	GTC	GCC	TTC	TAC	ATC	CCA	TTT	CTC		624
Pro	Tyr	Ala	Ile	Thr	Cys	Ser	Val	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu	
					195				200						205		
CTC	ATG	GTC	CTG	GCC	TAT	TAC	CGC	ATC	TAT	GTC	ACA	GCT	AAG	GAG	CAT		672
Leu	Met	Val	Leu	Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His		
					210			215							220		
GCC	CAT	CAG	ATC	CAG	ATG	TTA	CAA	CGG	GCA	GGA	GCC	TCC	TCC	GAG	AGC		720
Ala	His	Gln	Ile	Gln	Met	Leu	Gln	Arg	Ala	Gly	Ala	Ser	Ser	Glu	Ser		
					225			230				235					
AGG	CCT	CAG	TGC	GCA	GAC	CAG	CAT	AGC	ACT	CAT	CGC	ATG	AGG	ACA	GAG		768

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Arg	Pro	Gln	Ser	Ala	Asp	Gln	His	Ser	Thr	His	Arg	Met	Arg	Thr	Glu	
240						245					250					
ACC	AAA	GCA	GCC	AAG	ACC	CTG	TGC	ATC	ATC	ATG	GGT	TGC	TTC	TGC	CTC	816
Thr	Lys	Ala	Ala	Lys	Thr	Leu	Cys	Ile	Ile	Met	Gly	Cys	Phe	Cys	Leu	
255						260				265					270	
TGC	TGG	GCA	CCA	TTC	TTT	GTC	ACC	AAT	ATT	GTG	GAT	CCT	TTC	ATA	GAC	864
Cys	Trp	Ala	Pro	Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp	
						275			280					285		
TAC	ACT	GTC	CCT	GGG	CAG	GTC	TGG	ACT	GCT	TTC	CTC	TGG	CTC	GGC	TAT	912
Tyr	Thr	Val	Pro	Gly	Gln	Val	Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr	
						290		295				300				
ATC	AAT	TCC	GGG	TTG	AAC	CCT	TTT	CTC	TAC	GCC	TTC	TTG	AAT	AAG	TCT	960
Ile	Asn	Ser	Gly	Leu	Asn	Pro	Phe	Leu	Tyr	Ala	Phe	Leu	Asn	Lys	Ser	
						305		310			315					
TTT	AGA	CGT	GCC	TTC	CTC	ATC	ATC	CTC	TGC	TGT	GAT	GAT	GAG	CCC	TAC	1008
Phe	Arg	Arg	Ala	Phe	Leu	Ile	Ile	Leu	Cys	Cys	Asp	Asp	Glu	Arg	Tyr	
						320		325		330						
CGA	AGA	CCT	TCC	ATT	CTG	GGC	CAG	ACT	GTC	CCT	TGT	TCA	ACC	ACA	ACC	1056
Arg	Arg	Pro	Ser	Ile	Leu	Gly	Gln	Thr	Val	Pro	Cys	Ser	Thr	Thr	Thr	
					340				345				350			
ATT	AAT	GGA	TCC	ACA	CAT	GTA	CTA	AGG	GAT	GCA	GTC	GAG	TGT	GCT	GCC	1104
Ile	Asn	Gly	Ser	Thr	His	Val	Leu	Arg	Asp	Ala	Val	Glu	Cys	Gly	Gly	
					355			360			365					
CAG	TGG	GAG	AGT	CAG	TGT	CAC	CCG	CCA	GCA	ACT	TCT	CCT	TTG	GTG	GCT	1152
Gln	Trp	Glu	Ser	Gln	Cys	His	Pro	Pro	Ala	Thr	Ser	Pro	Leu	Val	Ala	
					370			375			380					
GCT	CAG	CCC	AGT	GAC	ACT	TAGCCCCCTG		GGACAAATGAC		CCAGAAAGACA						1200
Ala	Gln	Pro	Ser	Asp	Thr											
			385													
GGCATGCC		CGAAAGAGGG		CCAGGTCTTA		AGCTGCTCT		TGTGCGCGAC		TGCACCCGGC						1260
ATTCTCTTCA		CCTGAGGCTT		TCCGTCCCCC		AGTGCAGGAA		CCCGGTGCTC		GCTGGG						1316

(2) INFORMATION FOR SEQ ID NO:8:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 388 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Asp	Lys	Leu	Asp	Ala	Asn	Val	Ser	Ser	Glu	Glu	Gly	Phe	Gly	Ser
1				5						10					15
Val	Glu	Lys	Val	Val	Leu	Leu	Thr	Phe	Leu	Ser	Thr	Val	Ile	Leu	Met
				20				25					30		
Ala	Ile	Leu	Gly	Asn	Leu	Leu	Val	Met	Val	Ala	Val	Cys	Trp	Asp	Arg
			35				40					45			
Gln	Leu	Arg	Lys	Ile	Lys	Thr	Asn	Tyr	Phe	Ile	Val	Ser	Leu	Ala	Phe
			50			55					60				
Ala	Asp	Leu	Leu	Val	Ser	Val	Leu	Val	Met	Pro	Phe	Gly	Ala	Ile	Glu
65				70					75					80	
Leu	Val	Gln	Asp	Ile	Trp	Ile	Tyr	Gly	Glu	Val	Phe	Cys	Leu	Val	Arg
				85				90					95		
Thr	Ser	Leu	Asp	Val	Leu	Leu	Thr	Thr	Ala	Ser	Ile	Phe	His	Leu	Cys
				100			105						110		
Cys	Ile	Ser	Leu	Asp	Arg	Tyr	Tyr	Ala	Ile	Cys	Cys	Gln	Pro	Leu	Val
			115			120				125					
Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu	Gly	Gly
			130			135					140				
Cys	Trp	Val	Ile	Pro	Thr	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met	Gln	Gly
145					150				155					160	
Trp	Asn	Asn	Ile	Gly	Ile	Ile	Asp	Leu	Ile	Glu	Lys	Arg	Lys	Phe	Asn
				165				170					175		
Gln	Asn	Ser	Asn	Ser	Thr	Tyr	Cys	Val	Phe	Met	Val	Asn	Lys	Pro	Tyr
				180				185					190		

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Ala	Ile	Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu	Leu	Met	
195					200						205					
V	I	Leu	Al	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His	Ala	His
210					215						220					
Gln	Ile	Gln	Met	Leu	Gln	Arg	Ala	Gly	Ala	Ser	Ser	Glu	Ser	Arg	Pro	
225				230					235					240		
Gln	Ser	Ala	Asp	Gln	His	Ser	Thr	His	Arg	Met	Arg	Thr	Glu	Thr	Lys	
	245						250					255				
Ala	Ala	Lys	Thr	Leu	Cys	Ile	Ile	Met	Gly	Cys	Phe	Cys	Leu	Cys	Trp	
	260					265					270					
Ala	Pro	Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp	Tyr	Thr	
	275				280					285						
Val	Pro	Gly	Gln	Val	Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr	Ile	Asn	
290				295						300						
Ser	Gly	Leu	Asn	Pro	Phe	Leu	Tyr	Ala	Phe	Leu	Asn	Lys	Ser	Phe	Arg	
305				310					315					320		
Arg	Ala	Phe	Leu	Ile	Ile	Leu	Cys	Cys	Asp	Asp	Glu	Arg	Tyr	Arg	Arg	
	325						330					335				
Pro	Ser	Ile	Leu	Gly	Gln	Thr	Val	Pro	Cys	Ser	Thr	Thr	Thr	Ile	Asn	
	340					345					350					
Gly	Ser	Thr	His	Val	Leu	Arg	Asp	Ala	Val	Glu	Cys	Gly	Gly	Gln	Trp	
	355				360					365						
Glu	Ser	Gln	Cys	His	Pro	Pro	Ala	Thr	Ser	Pro	Leu	Val	Ala	Ala	Gln	
370				375						380						
Pro	Ser	Asp	Thr													
385																

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: modified\_base

(B) LOCATION: 14

(ix) FEATURE:

(A) NAME/KEY: modified\_base

(B) LOCATION: 20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGGAATTCTG YGYAATHKCA CTGGAYNGST A

31

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: modified\_base

(B) LOCATION: 4

(ix) FEATURE:

(A) NAME/KEY: modified\_base

(B) LOCATION: 7

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(ix) FEATURE:

- (A) NAME/KEY: modified\_base  
(B) LOCATION: 9..10

(ix) FEATURE:

- (A) NAME/KEY: modified\_base  
(B) LOCATION: 13

(ix) FEATURE:

- (A) NAME/KEY: modified\_base  
(B) LOCATION: 25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATAAVARA A ARAGGDAATRW ARAAAC

27

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCAAAAGCAT GATTCAGGG ACTCTGGTTC ATTGTGTATG GGCAA

45

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCTCAATCAG AAGCATGATT CCAGG

25

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTCGGTCTATA GGAACAAAGAT GACCC

25

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 792 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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## (vi) ORIGINAL SOURCE:

(F) TISSUE TYPE: Brain

## (vii) IMMEDIATE SOURCE:

(A) LIBRARY: HUMAN BRAIN

(B) CLONE: S10-87

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..783

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTG	GTC	TAT	AGG	AAC	AAG	ATG	ACC	CCT	CTG	CGC	ATC	GCA	TTA	ATG	CTG		48
Leu	Val	Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu		
1									10					15			
GGA	GGC	TGC	TGG	GTC	ATC	CCC	ACG	TTT	ATT	TCT	TTT	CTG	CCT	ATA	ATG		96
Gly	Gly	Cys	Trp	Val	Ile	Pro	Thr	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met		
									25				30				
CAA	GGC	TGG	AAT	AAC	ATT	GGC	ATA	ATT	GAT	TTG	ATA	GAA	AAG	AGG	AAG		144
Gln	Gly	Trp	Asn	Asn	Ile	Gly	Ile	Ile	Asp	Leu	Ile	Glu	Lys	Arg	Lys		
									40			45					
TTC	AAC	CAG	AAC	TCT	AAC	TCT	ACG	TAC	TGT	GTC	TTC	ATG	GTC	AAC	AAG		192
Phe	Asn	Gln	Asn	Ser	Asn	Ser	Thr	Tyr	Cys	Val	Phe	Met	Val	Asn	Lys		
									55			60					
CCC	TAC	GCC	ATC	ACC	TGC	TCT	GTG	GTG	GCC	TTC	TAC	ATC	CCA	TTT	CTC		240
Pro	Tyr	Ala	Ile	Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu		
									70			75		80			
CTC	ATG	GTG	CTG	GCC	TAT	TAC	CGC	ATC	TAT	GTC	ACA	GCT	AAG	GAG	CAT		288
Leu	Met	Val	Leu	Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His		
									85			90		95			
GCC	CAT	CAG	ATC	CAG	ATG	TTA	CAA	CGG	GCA	GGA	GCC	TCC	TCC	GAG	AGC		336
Ala	His	Gln	Ile	Gln	Met	Leu	Gln	Arg	Ala	Gly	Ala	Ser	Ser	Glu	Ser		
									100			105		110			
AGG	CCT	CAG	TCG	GCA	GAC	CAG	CAT	AGC	ACT	CAT	CGC	ATG	AGG	ACA	GAG		384
Arg	Pro	Gln	Ser	Ala	Asp	Gln	His	Ser	Thr	Mis	Arg	Met	Arg	Thr	Glu		
									115			120		125			

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ACC	AAA	GCA	GCC	AAG	ACC	CTG	TGC	ATC	ATC	ATC	ATG	GGT	TGC	TTC	TGC	CTC		432
Thr	Lys	Ala	Ala	Lys	Thr	Leu	Cys	Ile	Ile	Ile	Met	Gly	Cys	Phe	Cys	Leu		
130					135						140							
TGC	TGG	GCA	CCA	TTC	TTT	GTC	ACC	AAT	ATT	GTG	GAT	CCT	TTC	ATA	GAC		480	
Cys	Trp	Ala	Pro	Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp			
145					150					155					160			
TAC	ACT	GTC	CCT	GGG	CAG	GTG	TGG	ACT	GCT	TTC	CTC	TGG	CTC	GGC	TAT		528	
Tyr	Thr	Val	Pro	Gly	Gln	Val	Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr			
					165				170					175				
ATC	AAT	TCC	GGG	TTG	AAC	CCT	TTT	CTC	TAC	GCC	TTC	TTG	AAT	AAG	TCT		576	
Ile	Asn	Ser	Gly	Leu	Asn	Pro	Phe	Leu	Tyr	Ala	Phe	Leu	Asn	Lys	Ser			
					180				185					190				
TTT	AGA	CGT	GCC	TTC	CTC	ATC	ATC	CTC	TGC	TGT	GAT	GAT	GAG	CGC	TAC		624	
Phe	Arg	Arg	Ala	Phe	Leu	Ile	Ile	Leu	Cys	Cys	Asp	Asp	Glu	Arg	Tyr			
					195			200			205							
CGA	AGA	CCT	TCC	ATT	CTG	GGC	CAG	ACT	GTC	CCT	TGT	TCA	ACC	ACA	ACC		672	
Arg	Arg	Pro	Ser	Ile	Leu	Gly	Gln	Thr	Val	Pro	Cys	Ser	Thr	Thr	Thr			
					210			215			220							
ATT	AAT	GGA	TCC	ACA	CAT	GTA	CTA	AGG	TAC	ACC	CTT	CTG	CAC	AGG	GGA		720	
Ile	Asn	Gly	Ser	Thr	His	Val	Leu	Arg	Tyr	Thr	Val	Leu	His	Arg	Gly			
					225			230			235				240			
CAT	CAT	CAG	GAA	CTC	GAG	AAA	CTG	CCC	ATA	CAC	AAT	GAC	CCA	GAA	TCC		768	
Mis	Mis	Gln	Glu	Leu	Glu	Lys	Leu	Pro	Ile	Mis	Asn	Asp	Pro	Glu	Ser			
					245			250			255							
CTG	GAA	TCA	TGC	TTC		TGATTGAGG											792	
Leu	Glu	Ser	Cys	Phe														
				260														

(2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 261 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Leu	Val	Tyr	Arg	Asn	Lys	Met	Thr	Pro	Leu	Arg	Ile	Ala	Leu	Met	Leu
1				5					10					15	
Gly	Gly	Cys	Trp	Val	Ile	Pro	Thr	Phe	Ile	Ser	Phe	Leu	Pro	Ile	Met
								20			25			30	
Gln	Gly	Trp	Asn	Asn	Ile	Gly	Ile	Ile	Asp	Leu	Ile	Glu	Lys	Arg	Lys
								35			40			45	
Phe	Asn	Gln	Asn	Ser	Asn	Ser	Thr	Tyr	Cys	Val	Phe	Met	Val	Asn	Lys
								50			55			60	
Pro	Tyr	Ala	Ile	Thr	Cys	Ser	Val	Val	Ala	Phe	Tyr	Ile	Pro	Phe	Leu
								65			70			75	
Leu	Met	Val	Leu	Ala	Tyr	Tyr	Arg	Ile	Tyr	Val	Thr	Ala	Lys	Glu	His
								85			90				95
Ala	His	Gln	Ile	Gln	Met	Leu	Gln	Arg	Ala	Gly	Ala	Ser	Ser	Glu	Ser
								100			105			110	
Arg	Pro	Gln	Ser	Ala	Asp	Gln	His	Ser	Thr	His	Arg	Met	Arg	Thr	Glu
								115			120			125	
Thr	Lys	Ala	Ala	Lys	Thr	Leu	Cys	Ile	Ile	Met	Gly	Cys	Phe	Cys	Leu
								130			135			140	
Cys	Trp	Ala	Pro	Phe	Phe	Val	Thr	Asn	Ile	Val	Asp	Pro	Phe	Ile	Asp
								145			150				160
Tyr	Thr	Val	Pro	Gly	Gln	Val	Trp	Thr	Ala	Phe	Leu	Trp	Leu	Gly	Tyr
								165			170			175	
Ile	Asn	Ser	Gly	Leu	Asn	Pro	Phe	Leu	Tyr	Ala	Phe	Leu	Asn	Lys	Ser
								180			185			190	
Phe	Arg	Arg	Ala	Phe	Leu	Ile	Ile	Leu	Cys	Cys	Asp	Asp	Glu	Arg	Tyr
								195			200			205	
Arg	Arg	Pro	Ser	Ile	Leu	Gly	Gln	Thr	Val	Pro	Cys	Ser	Thr	Thr	Thr
								210			215			220	
Ile	Asn	Gly	Ser	Thr	Mis	Val	Leu	Arg	Tyr	Thr	Val	Leu	His	Arg	Gly

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225		230		235		240									
His	His	Gln	Glu	Leu	Glu	Lys	Leu	Pro	Ile	His	Asn	Asp	Pro	Glu	Ser
				245					250					255	
Leu	Glu	Ser	Cys	Phe											
				260											

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What is claimed is:

1. An isolated nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor.
- 5 2. A nucleic acid molecule of claim 1, wherein the nucleic acid molecule encodes a human 5-HT<sub>4</sub> receptor.
- 10 3. A nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a DNA molecule.
- 15 4. A DNA molecule of claim 3, wherein the DNA molecule is a cDNA molecule.
- 20 5. A DNA molecule of claim 3 wherein the DNA molecule is genomic DNA.
6. A nucleic acid molecule of claim 2, wherein the nucleic acid molecule is a DNA molecule.
- 25 7. An isolated DNA molecule encoding a mammalian 5-HT<sub>4</sub> receptor having the sequence H<sub>2</sub>N-Y-X-COOH wherein Y is the amino acid sequence beginning at amino acid 1 and ending at amino acid 359 of Figure 1 (SEQ ID NO. 2) and wherein X is an amino acid sequence encoding the carboxy terminal region of the receptor.
- 30 8. An isolated nucleic acid molecule of claim 7, wherein X is the amino acid sequence beginning at amino acid 360 and ending at amino acid 387 of Figure 1 (SEQ ID NO. 2).
- 35 9. An isolated nucleic acid molecule of claim 7, wherein X is the amino acid sequence beginning at

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amino acid 360 and ending at amino acid 406 of  
Figure 2 (SEQ ID NO. 4).

10. An isolated nucleic acid molecule of claim 7,  
5 wherein Y is encoded by the nucleotide sequence  
from nucleotide 101 to nucleotide 1177 of Figure  
1 (SEQ ID NO. 1).
11. An isolated nucleic acid molecule of claim 8,  
10 wherein X is encoded by the nucleotide sequence  
from nucleotide 1178 to nucleotide 1261 of  
Figure 1 (SEQ ID NO. 1).
12. An isolated nucleic acid molecule of claim 9,  
15 wherein X is encoded by the nucleotide sequence  
from nucleotide 1127 to nucleotide 1267 of Figure  
2 (SEQ ID NO. 3).
13. A vector comprising a cDNA molecule of claim 4.  
20
14. A plasmid vector of claim 13.
15. A vector of claim 13 adapted for expression in a  
25 bacterial cell which comprises regulatory elements  
necessary for expression of the cDNA encoding a 5-  
HT<sub>4</sub> receptor in the bacterial cell operatively  
linked to the cDNA encoding the 5-HT<sub>4</sub> receptor as  
to permit expression thereof.
30. 16. A vector of claim 13 adapted for expression in a  
yeast cell which comprises the regulatory elements  
necessary for the expression of the cDNA encoding  
a 5-HT<sub>4</sub> receptor in the yeast cell operatively  
linked to the cDNA encoding the 5-HT<sub>4</sub> receptor as  
35 to permit expression thereof.
17. A vector of claim 13 adapted for expression in an

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insect cell which comprises the regulatory elements necessary for expression of the cDNA encoding a 5-HT<sub>4</sub> receptor in the insect cell operatively linked to the cDNA encoding the 5-HT<sub>4</sub> receptor as to permit expression thereof.

5

18. A vector of claim 13 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the cDNA encoding a 5-HT<sub>4</sub> receptor in the mammalian cell operatively linked to the cDNA encoding the 5-HT<sub>4</sub> receptor as to permit expression thereof.
- 10
19. A plasmid of claim 14 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell operatively linked to the DNA encoding a 5-HT<sub>4</sub> receptor as to permit expression thereof.
- 15
20. A plasmid of claim 19 designated pcEXV-S10-87 (ATCC Accession No. 75390).
- 20
21. A plasmid of claim 19 designated pcEXV-S10-95 (ATCC Accession No. 75391).
- 25
22. A plasmid of claim 19 designated pBluescript-hS10 (ATCC Accession No. 75392).
23. A mammalian cell comprising the plasmid of claim 14.
- 30
24. A mammalian cell of claim 23, wherein the mammalian cell is an LM (tk-) cell.
- 35
25. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of

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specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor.

5        26. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human 5-HT<sub>4</sub> receptor.

10        27. The nucleic acid probe of claim 25, wherein the nucleic acid is DNA.

15        28. The nucleic acid probe of claim 26, wherein the nucleic acid is DNA.

20        29. A mixture of nucleic acid probes in accordance with claim 25, such probes having sequences which differ from one another at predefined positions.

25        30. An antisense oligonucleotide having a sequence capable of specifically binding to a mRNA molecule encoding a mammalian 5-HT<sub>4</sub> receptor so as to prevent translation of the mRNA molecule.

30        31. An antisense oligonucleotide capable of specifically binding to a mRNA molecule encoding a human 5-HT<sub>4</sub> receptor so as to prevent translation of the mRNA molecule.

35        32. An antisense oligonucleotide of claim 30 comprising chemical analogs of nucleotides.

35        33. A mixture of antisense oligonucleotides according to claim 30, such oligonucleotides having sequences which differ from one another at

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predefined positions.

34. A method for detecting expression of a mammalian 5-HT<sub>4</sub> receptor, which comprises obtaining RNA from cells or tissue, contacting the RNA so obtained with a nucleic acid probe of claim 25 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of the mammalian 5-HT<sub>4</sub> receptor, and thereby detecting the expression of the mammalian 5-HT<sub>4</sub> receptor.
- 5
35. A method for detecting expression of a human 5-HT<sub>4</sub> receptor, which comprises obtaining RNA from cells or tissue, contacting the RNA so obtained with a nucleic acid probe of claim 26 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of the human 5-HT<sub>4</sub> receptor, and thereby detecting the expression of the human 5-HT<sub>4</sub> receptor.
- 10
36. A method of detecting expression of a mammalian 5-HT<sub>4</sub> receptor in a cell or tissue by in situ hybridization, which comprises contacting the cell or tissue with a nucleic acid probe of claim 25 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of a mammalian 5-HT<sub>4</sub> receptor, and thereby detecting the expression of a mammalian 5-HT<sub>4</sub> receptor.
- 15
- 30
- 35
37. A method of detecting expression of a human 5-HT<sub>4</sub> receptor in a cell or tissue by in situ hybridization, which comprises contacting the cell

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or tissue with a nucleic acid probe of claim 26 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of a human 5-HT<sub>4</sub> receptor, and thereby detecting the expression of the human 5-HT<sub>4</sub> receptor.

- 10        38. A method of isolating from a gene library a gene  
          encoding a receptor other than the 5-HT<sub>2</sub> receptor  
          which comprises contacting the library under  
          hybridizing conditions with a probe of claim 27  
          and isolating any gene to which the probe  
          hybridizes.

15        39. A method of claim 38, which additionally comprises  
          simultaneously contacting the DNA comprising the  
          library under hybridizing conditions with a second  
          nucleic acid probe comprising a sequence capable  
          of hybridizing to a DNA sequence of the  
          complementary strand of the DNA of the gene to  
          which the first probe hybridizes, treating any  
          gene sequence to which both probes hybridized so  
          as to produce multiple copies of the gene  
          sequence, isolating the amplified gene sequence  
          and using the isolated gene sequence as a probe to  
          isolate from a gene library the gene to which the  
          amplified DNA sequence hybridizes.

20        40. The gene isolated by the method of claim 38 or 39.

25        41. A synthetic gene which comprises the isolated  
          nucleic acid molecule of claim 1 and at least one  
          regulatory element attached thereto so as to  
          increase the number of RNA molecules transcribed  
          from the synthetic gene.

30

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42. A synthetic gene which comprises the isolated nucleic acid molecule of claim 1 and at least one regulatory element attached thereto so as to decrease the number of RNA molecules transcribed from the synthetic gene.
- 5
43. An isolated mammalian 5-HT<sub>4</sub> receptor protein.
- 10
44. The receptor protein of claim 43, wherein the mammalian 5-HT<sub>4</sub> receptor protein is a human 5-HT<sub>4</sub> receptor.
- 15
45. A method of preparing a mammalian 5-HT<sub>4</sub> receptor of claim 43, which comprises inducing cells to express the mammalian 5-HT<sub>4</sub> receptor and recovering the mammalian 5-HT<sub>4</sub> receptor from the resulting cells.
- 20
46. A method of preparing a mammalian 5-HT<sub>4</sub> receptor protein of claim 43, which comprises inserting a nucleic acid molecule encoding the mammalian 5-HT<sub>4</sub> receptor in a suitable vector, inserting the resulting vector in suitable host cell and recovering the mammalian 5-HT<sub>4</sub> receptor produced by the resulting cell.
- 25
47. A method of preparing a human 5-HT<sub>4</sub> receptor of claim 44, which comprises inducing cells to express the human 5-HT<sub>4</sub> receptor and recovering the human 5-HT<sub>4</sub> receptor from the resulting cells.
- 30
48. A method of preparing a human 5-HT<sub>4</sub> receptor protein of claim 44, which comprises inserting a nucleic acid molecule encoding the human 5-HT<sub>4</sub> receptor in a suitable vector, inserting the resulting vector in suitable host cell and recovering the human 5-HT<sub>4</sub> receptor produced by
- 35

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the resulting cell.

49. An antibody directed to a mammalian 5-HT<sub>4</sub> receptor or to a protein fragment of the mammalian 5-HT<sub>4</sub> receptor.
- 5 50. An antibody directed to a human 5-HT<sub>4</sub> receptor or to a protein fragment of the human 5-HT<sub>4</sub> receptor.
- 10 51. An antibody of claim 49, wherein the antibody is a monoclonal antibody.
- 15 52. An antibody of claim 50, wherein the antibody is a monoclonal antibody.
- 20 53. A monoclonal antibody of claim 51, wherein the antibody is directed to an epitope of a mammalian cell-surface 5-HT<sub>4</sub> receptor and having an amino acid sequence substantially the same as the amino acid sequence of a cell-surface epitope of the mammalian 5-HT<sub>4</sub> receptor.
- 25 54. A monoclonal antibody of claim 52, wherein the antibody is directed to an epitope of a human cell-surface 5-HT<sub>4</sub> receptor and having an amino acid sequence substantially the same as the amino acid sequence for a cell-surface epitope of the human 5-HT<sub>4</sub> receptor.
- 30 55. A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human 5-HT<sub>4</sub> receptor and a pharmaceutically acceptable carrier.
- 35 56. A pharmaceutical composition comprising an amount of a substance effective to alleviate

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abnormalities resulting from underexpression of a human 5-HT<sub>4</sub> receptor and a pharmaceutically acceptable carrier.

- 5        57. A pharmaceutical composition comprising an effective amount of an oligonucleotide of claim 31 effective to reduce expression of a human 5-HT<sub>4</sub> receptor by passing through a cell membrane and specifically binding with mRNA encoding a human 5-HT<sub>4</sub> receptor in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane.
- 10
- 15        58. A pharmaceutical composition claim 57, wherein the nucleotide is coupled to a substance which inactivates mRNA.
- 20        59. A pharmaceutical composition of claim 58, wherein the substance which inactivates the mRNA is a ribozyme.
- 25
- 30        60. A pharmaceutical composition of claim 58, wherein the pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane comprises a structure which binds to a transporter specific for a selected cell type and is thereby taken up by the cells of the selected cell type.
- 35        61. A pharmaceutical composition which comprises an amount of the antibody of claim 50 effective to block binding of naturally occurring substrates to a human 5-HT<sub>4</sub> receptor and a pharmaceutically acceptable carrier.
62. A transgenic nonhuman mammal which comprises a nucleic acid molecule of claim 1.

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63. A transgenic nonhuman mammal whose genome comprises a nucleic acid molecule of claim 1 so placed as to be transcribed into antisense mRNA complementary to mRNA encoding a human 5-HT<sub>4</sub> receptor and which hybridizes to mRNA encoding a human 5-HT<sub>4</sub> receptor thereby reducing its translation.
- 5
64. The transgenic nonhuman mammal of claim 62, wherein the nucleic acid molecule further comprises an inducible promoter.
- 10
65. The transgenic nonhuman mammal of claim 62 or 64 wherein the nucleic molecule additionally comprises tissue specific regulatory elements.
- 15
66. The transgenic non-human mammal of 62 wherein the transgenic non-human mammal is a mouse.
- 20
67. A method for determining the physiological effects of varying the levels of expression of a human 5-HT<sub>4</sub> receptor which comprises producing a transgenic non-human mammal whose levels of expression of a human 5-HT<sub>4</sub> receptor can be varied by use of an inducible promoter.
- 25
68. A method for determining the physiological effects of expressing varying levels of a human 5-HT<sub>4</sub> receptor which comprises producing a panel of transgenic non-human mammals each expressing a different amount of a human 5-HT<sub>4</sub> receptor.
- 30
69. A method for determining whether a compound not known to be capable of specifically binding to a human 5-HT<sub>4</sub> receptor can specifically bind to the human 5-HT<sub>4</sub> receptor, which comprises contacting a mammalian cell comprising a plasmid adapted for
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expression in a mammalian cell which plasmid further comprises DNA which expresses a human 5-HT<sub>4</sub> receptor on the cell's surface with the compound under conditions permitting binding of ligands known to bind to a human 5-HT<sub>4</sub> receptor, detecting the presence of any compound bound to the human 5-HT<sub>4</sub> receptor, the presence of bound compound indicating that the compound is capable of specifically binding to the human 5-HT<sub>4</sub> receptor.

- 15        70. The method of claim 70, wherein the mammalian cell  
            is a non-neuronal cell.

20        71. A method of screening compounds to identify drugs  
            which interact with, and specifically bind to, a  
            human 5-HT<sub>4</sub> receptor on the surface of a cell,  
            which comprises contacting a mammalian cell which  
            comprises a plasmid adapted for expression in a  
            mammalian cell which plasmid further comprises DNA  
            which expresses a human 5-HT<sub>4</sub> receptor on the  
            cell's surface with a plurality of compounds,  
            determining those compounds which bind to the  
            human 5-HT<sub>4</sub> receptor expressed on the cell surface  
            of the mammalian cell, and thereby identifying  
            compounds which interact with, and specifically  
            bind to, the human 5-HT<sub>4</sub> receptor.

25        72. The method of claim 71, wherein the mammalian cell  
            is a non-neuronal cell.

30        73. A method for determining whether a compound not  
            known to be capable of specifically binding to a  
            human 5-HT<sub>4</sub> receptor can specifically bind to a  
            human 5-HT<sub>4</sub> receptor, which comprises preparing a  
            cell extract from mammalian cells, which comprise  
            a plasmid adapted for expression in a mammal,

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which plasmid further comprises DNA which expresses a human 5-HT<sub>4</sub> receptor on the cell's surface, isolating a membrane fraction from the cell extract, incubating the compound with the membrane fraction under conditions permitting binding of ligands known to bind to the human 5-HT<sub>4</sub> receptor, detecting the presence of any bound compound, and thereby determining whether the compound is capable of specifically binding to the human 5-HT<sub>4</sub> receptor.

- 10
  - 15
  - 20
  - 25
  - 30
  - 35
74. The method of claim 73, wherein the mammalian cell is a non-neuronal cell.
75. A method for screening compounds to identify drugs that interact with, and specifically bind to, a human 5-HT<sub>4</sub> receptor, which comprises preparing a cell extract from mammalian cells, which comprise a plasmid adapted for expression in a mammalian cell which plasmid further comprises DNA which expresses a human 5-HT<sub>4</sub> receptor on the cell's surface, isolating a membrane fraction from the cell extract, incubating the membrane fraction with a plurality of compounds, determining those compounds which interact with and bind to the human 5-HT<sub>4</sub> receptor, and thereby identifying compounds which interact with, and specifically bind to, the human 5-HT<sub>4</sub> receptor.
76. The method of claim 75, wherein the mammalian cell is a non-neuronal cell.
77. A method for identifying a compound which is not known to be capable of binding to the human 5-HT<sub>4</sub> receptor activates the human 5-HT<sub>4</sub> receptor on the surface of a mammalian cell or prevents a ligand which does so, which comprises contacting the

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mammalian cell which cell comprises a plasmid adapted for expression in the mammalian cell such plasmid further comprising DNA which expresses the human 5-HT<sub>4</sub> receptor on the cell surface of the mammalian cell with the compound under conditions permitting activation of blockade of a functional response, determining whether the compound activates the human 5-HT<sub>4</sub> receptor or prevents a ligand which does so, and thereby identifying the compound as a compound which interacts with, and activates the human 5-HT<sub>4</sub> receptor or prevents the activation of the human 5-HT<sub>4</sub> receptor by a ligand which does so.

- 15 78. The method of claim 77, wherein the mammalian cell is a non-neuronal cell comprising the cellular components necessary to produce a second messenger and wherein the determination of whether the compound activates or blocks the activation of the human 5-HT<sub>4</sub> comprises detecting the change in the concentration of the second messenger.
- 20 79. The method of claim 78, wherein the second messenger is cyclic AMP (cAMP).
- 25 80. The method of claim 78, wherein the non-neuronal cell is a COS-7 cell.
- 30 81. A method of claim 78, wherein the second messenger is an inositol phosphate metabolite.
82. The method of claim 78, wherein the second messenger is intracellular calcium.
- 35 83. A compound identified by the method of claim 69, 73 or 77.

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84. A pharmaceutical composition of a drug identified by the method of claim 71 or 75.
- 5        85. A method for detecting the presence of a human 5-HT<sub>4</sub> receptor on the surface of a cell, which comprises contacting the cell with an antibody of claim 50, under conditions that permit binding of the antibody to the receptor, detecting the presence of any of the antibody bound to the cell, and thereby the presence of a human 5-HT<sub>4</sub> receptor on the surface of the cell.
- 10      86. A method for treating an abnormal condition related to an excess of activity of a human 5-HT<sub>4</sub> receptor, which comprises administering a patient an amount of a pharmaceutical composition of claim 84, effective to reduce 5-HT<sub>4</sub> activity as a result of naturally occurring substrate binding to and activating the 5-HT<sub>4</sub> receptor.
- 15      87. The method of treating abnormalities which are alleviated by an increase in the activity of a 5-HT<sub>4</sub> receptor, which comprises administering a patient an amount of a pharmaceutical composition of claim 84, effective to increase the activity of the 5-HT<sub>4</sub> receptor thereby alleviating abnormalities resulting from abnormally low receptor activity.
- 20      88. A method for diagnosing a predisposition to a disorder associated with the expression of a human 5-HT<sub>4</sub> receptor allele which comprises:
- 25            a. obtaining DNA from subjects suffering from a disorder;
- 30            b. performing a restriction digest of the DNA
- 35

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with a panel of restriction enzymes;

c. electrophoretically separating the resulting DNA fragments on a sizing gel;

5

d. contacting the gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human 5-HT<sub>4</sub> receptor and labelled with a detectable marker;

10

e. detecting the labelled bands which have hybridized to the DNA encoding 5-HT<sub>4</sub> receptor, labelled with the detectable marker to create a unique band pattern specific to the DNA of subjects suffering with the disorder;

15

f. preparing DNA for diagnosis by steps a-e;

20

g. comparing the unique band pattern specific to the DNA of patients suffering from the disorder from step e and DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

25

89. The method of claim 88, wherein a disorder is associated with the expression of a specific human 5-HT<sub>4</sub> receptor allele is diagnosed.

30

90. A method of identifying a substance capable of alleviating the abnormalities resulting from overexpression of a human 5-HT<sub>4</sub> receptor which comprises administering a substance to the transgenic non-human mammal of claim 67 or 68, and determining whether the substance alleviates the

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physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of the human 5-HT<sub>4</sub> receptor.

- 5        91. A method of identifying a substance capable of alleviating the abnormalities resulting from underexpression of a human 5-HT<sub>4</sub> receptor, which comprises administering a substance to the transgenic mammal of claim 62, and determining  
10      whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of the human 5-HT<sub>4</sub> receptor.
- 15        92. A method of treating abnormalities in a subject, wherein the abnormality is alleviated by the reduced expression of a human 5-HT<sub>4</sub> receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim  
20      55, 57, 83 or 84 effective to reduce expression of the human 5-HT<sub>4</sub> receptor.
- 25        93. A method of treating abnormalities resulting from underexpression of a human 5-HT<sub>4</sub> receptor which comprises administering to a subject an amount of a pharmaceutical composition of claim 56, 83 or 84 effective to alleviate abnormalities resulting from underexpression of the human 5-HT<sub>4</sub> receptor.

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FIGURE 1-1

FIGURE 1-1, 1/32
FIGURE 1-2, 2/32

1	AGCCCTGGCGAGCCTGGCTTGGTCAAGGAGGATGCTCTGGGTGCCCAAGGGCTTG	60
61	TGGGCACATCACAAACGTACTCATGCCCATTTCTGTAATGGACAGACTTGATGCTAA	120
	M D R L D A N	7
121	TGTGAGTTCAACGAGGTTTCGGGTCTGGAGAAGGTGTACTGGCTCACGTTCTTCGC	180
8	V S S N E G F G S V E K V V L L T F F A	27
181	AATGGTTATCCCTGATGGCCATCCCTGGCAACCTGGCTGGTGAATGGTGGCTGAGGGA	240
28	M V I L M A I L G N L L V M V A V C R D	47
241	CAGGCAGCTCAGGAAAATAAAACCATTATTTCATTGGTCTCTGCCTTGCCTGATCT	300
48	R Q L R K I K T N Y F I V S L A F A D L	67
301	GCTGGTTTCCGGTGGTGAATGCCCTTCGGCATTGGATGGCTCAAGACATCTGGTT	360
68	L V S V L V N A F G A I E L V Q D I W F	87
361	TTATGGGGatgttttgcctgggtccggacctctcgatgtcctactcaccacagcatc	420
88	Y G E M F C L V R T S L D V L L T A S	107
421	attttttcacccctctgtcgatttccctggataggtaattatGCCATCTGGCTGTCACCTT	480
108	I F H L C C I S L D R Y Y A I C C Q P L	127
481	GGTTTATAGAAACAAGATGACCCCTCTACGGCATGCCATTAAATGCTGGGAGGGCTGGGT	540
128	V Y R N K M T P L R I A L M L G G C W V	147
541	CATTCCCATGTTTATATCTTTCTCCCCATAATGCAAGGCTGGAAACATGGCATAGT	600
148	I P M F I S F L P I M Q G W N N I G I V	167
601	TGATGTGATAGAGAAAGGAATTCAACCAACTCTAACCTCTACATTCTGTGTCTTCAT	660
168	D V I E K R K F N H N S N S T F C V F M	187
661	GGTCAACAAAGCCCTATGCCATCACCTGGCTCTGGGGCCCTCTACATCCGGTTCTCCCT	720
188	V N K P Y A I T C S V A F Y I P F L L	207

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FIGURE 1-2

721	CATGGTGCCTTACCGTATTCGGTACTGTCAAGGAGCATGCCAGGATCCA	780
208	M V L A Y Y R I Y V T A K E H A Q Q 1 Q	227
781	GATGTTACAACGGCAGGAGCCACCTCTGAAAGCAGGGCCCAGACAGCTGACCAGCACAG	840
228	M L Q R A G A T S E S R P Q T A D Q H S	247
841	CACACATGGCATGGGACAGAGACCCAAAGCAGGAGCAAGACTTTATGGTCATCATGGGCTG	900
248	T H R M R T E T K A A K T L C V 1 M G C	267
901	CTTCTGTTCTGGCCCCCTCTTGTCACCAATTATGGACCCCTTATAGACTA	960
268	F C F C W A P F V T N I V D P F 1 D Y	287
961	CACIGGCCGAGGGTGTGGACTGCTTCTGGCTTCTGGCTTATCAATTTCAGGGTT	1020
288	T V P E K V W T A F L W L G Y I N S G L	307
1021	GAACCCCTTTCTCTATGGCTTCTGAATAAGTCCTTCAGACGTCCTTCCTTATCATCCCT	1080
308	N P F L Y A F L N K S F R R A F L I I L	327
1081	CTGCTGTGATGAGGGCTACAAAAGACCCCCATTCTGGGGCAGAGACTGTCCCCCTGTTC	1140
328	C C D D E R Y K R P P I L G Q T V P C S	347
1141	AACACAAACCTTAATGGATCCACTCATGTGCTAAGGTATACAGTTTGCAATAGGGTCA	1200
348	T T I N G S T H V L R Y T V L H S G Q	367
1201	ACACCAAGGAACCTGGAAACTGGCCATACACAAATGACCCAGAGTCCCTGGAAICATGCTT	1260
368	H Q E L E K L P I H N D P E S L E S C F	387
1261	TTGATTGAAGACGTGGCTTGGCTTACCGGTTACATCCCATTGCAATGAAACAGGT	1320
*		
1321	TACTATGGAAATCACTCCTGACTCTGGGATCACCAAGTGAAGCATGAGCATGGTGAGGGAG	1380
1381	GGTCCGGTGAAGGTGACAGAGGACAGCATTTGAGTGGGACCTGAACCCAGCACATTAGG	1440
1441	ATTTCAGAACCGTGTGGGATTGAGATGTCATCAGACCCAGTGTCTTACCCAGGCCA	1500
1501	ACTGGCACCCATTCACCGCTGACATGTTGGTCAGTCCTTGCTCACACCTCTCCAGGG	1560
1561	CAGGAGCTGACTACCTCCTAATGTTGGGGAGCTCTTAAATTGTTGGAAGTTCACTGAT	1620
1621	TCATTGGGGACAGTCTCGCTG 1642	

FIGURE 2-1

1	CAGGCCACGGCAGGGCAGAACGCGCTCTGGCTAAAGGTGGCCAGCGATCTGCC	60
61	AGGGTGCCTCTCCCTTAGCCCTINCGAGCCGGTGGGGCTGGAGTCokGCCACGAGA	120
121	TCAAGAACCCCAGGGCCACGTGTGAAGGGTCCCTGTGGCACTGACATCCAACGTACTCA	180
181	TGCCCATTTCTGTAAATGGACAGACTTGATGCTAAATGTGAGTTCAACGAGGGTTCCGGG	240
	M D R L D A N V S S N E G F G	15
241	TCTGGAGAAGGTGCTCACGTCTGGTATGGCAATGGTTATCCTGATGCCATCCCTG	300
16	S V E K V V L L T F F A M V I L M A I L	35
301	GGCAAACCTGCTGGTGAATGGTGGCTGGGGACAGGGCAGCTAGGGAAAATAAAACC	360
36	G N L L V M V A V C R D R Q L R K I K	55
361	AATTATTCATTGGTCTCTGGCTTGGCTGATCTGGTGGTGAATGCC	420
56	N Y F I V S L A F A D L L V S V L V N A	75
421	TTGGTGCCTATTGAGTTGGTCAAGACATCTGGTTTATGGGAGATGTTGGCTGGTC	480
76	F G A I E L V Q D I W F Y G E H F C L V	95
481	CGGACCTCTGGATGGCTTACCTACCCACAGCATCAATTTCACCTCTGCTGCCCTTCC	540
96	R T S L D V L L T T A S I F H L C C I S	115
541	CTGGATAGGTATTGGCATCTGGCTGCAACCTTGGTTTATAGAACAGATGACCCCT	600
116	L D R Y Y A I C C Q P L V Y R N K M T P	135
601	CTACGGCATCGCATTAAATGGGGGGCTGGCTGGCATTCCTCATGTTTATCTTTCTC	660
136	L R I A L M L G G C V V I P M F I S F L	155
661	CCCATAATGCCAAGGCTGGAACAAACATCGGCATAGTTGATGATGAGAACATTTC	720
156	P I M Q G V N N I G I V D V I E K R K F	175
721	ACCACAACTCTAACTCTACATCTGGCTTCAACAGGCCCTATGCCATCAC	780
176	N H N S N S T F C V F M V N K P Y A I T	195
781	TGCTCTGGGGCCCTCTACATCCGGTTCTCCATGGTGGCTTATTACCGTATC	840
196	C S V V A F Y I P F L L M V L A Y Y R I	215

FIGURE 2-1, 3/32
FIGURE 2-2, 4/32

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FIGURE 2-2

841 TAIGTCACTGGCATGGGAGATGCCAGATCCAGATGTACAAACGGGAGGGCCACC 900  
 216 Y V T A K E H A Q Q I Q M L Q R A G A T 235  
 901 TCTGAAGCAGGCCAGACAGCTGACCCACAGCACACATCGCATGGGACAGAGACC 960  
 236 S E S R P Q T A D Q H S T H R M R T E T 255  
 961 AAAGCAGCCAAAGACTTTAATGIGTCATCATGGGCATTCATGCTTGCTGGCCCCITC 1020  
 256 K A A K T L C V I M G C F C F C W A P F 275  
 1021 TTTGTCACCAAATTGGACCCCTTICATAGACTACACTGTCAGGAGGGTGTGGACT 1080  
 276 F V T N I V D P F I D Y T V P E K V W T 295  
 1081 GCTTCCCTGGCTTGGCTATAATCAATTCAATTCAATTCAATTCAATTCAATTCAATT 1140  
 296 A F L W L G Y I N S G L N P F L Y A F L 315  
 1141 ATAAGTCTTTCAGACGGTGCCTTCCTTATCATCCTCTGCTGTGAIGATGAGGGCTACAA 1200  
 316 N K S F R R A F L I I L C C D D E R Y K 335  
 1201 AGACCCCCCATTCTGGGCCAGACTGTCCCCTGTCAACCACCAACCATTAAATGGATCCACT 1260  
 336 R P P I L G Q T V P C S T T I N G S T 355  
 1261 CATGTGCTAAGGGATAACAGTGGAAATGGGAGAGTGGGTGTACCTCACAA 1320  
 356 H V L R D T V E C G G Q W E S R C H L T 375  
 1321 GCAACTTCTTGGCTGGCTGCTCAGCCAGTGTACGTAACGTAAGGGCCAGGACAATGACTA 1380  
 376 A T S P L V A Q P V I R R P Q D N D L 395  
 1381 GAAGACAGCTGTAGCTTGAAGAAGCCAGTCCTAACGCTGCTACTTCGGTGTATGTGGCT 1440  
 396 E D S C S L K R S Q S \* 406  
 1441 GCCCTGGCACTTGTCTCCAAAGGCTTCCAAAGGCAATCCACCCCTGGACTT 1500  
 1501 CCCGCCACGATTCAGGGGIAATTAGGAAAGTCAGGGAGAGAAGGGCTTCCTCC 1560  
 1561 TAGCTTTCTGTTCACATTTCCTTCACCTTCCTGGAGTCCTCCACTCTGGGTCTC 1620  
 1621 TGAAGTCCACGACCCAGTCCCCTTTCAGTCAGTCCTGCTTACCGT 1680  
 1681 GTTGAATTTCAGTTCCAAACATGCCCTCTTGAAGTGTCACTTACGATACTGTCAA 1740  
 1741 ACATGTGCCCTGCTTGTACACTTCTT 1768

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## FIGURE 3

1 MDRLDANVSSNEGFGSVEKVLLTFFAMVILMAILGNLLVMVAVCRDRQL 50  
|||||  
1 MDRLDANVSSNEGFGSVEKVLLTFFAMVILMAILGNLLVMVAVCRDRQL 50  
  
51 RKIKTNYFIVSLAFADLLVSVLVNAFGAIELVQDIWFYGEMFCLVRTSLD 100  
|||||  
51 RKIKTNYFIVSLAFADLLVSVLVNAFGAIELVQDIWFYGEMFCLVRTSLD 100  
  
101 VLLTASIFHLCCISLDRYYAICCQPLVYRNKMTPLRIALMLGGCWIPM 150  
|||||  
101 VLLTASIFHLCCISLDRYYAICCQPLVYRNKMTPLRIALMLGGCWIPM 150  
  
151 FISFLPIMQGWNNIGIVDVIEKRKFHNHSNSTFCVFMVNKPYAITCSVVA 200  
|||||  
151 FISFLPIMQGWNNIGIVDVIEKRKFHNHSNSTFCVFMVNKPYAITCSVVA 200  
  
201 FYIPFLLMVLAYYRIYVTAKEHQQIQMLQRAGATSESRPQTADQHSTHR 250  
|||||  
201 FYIPFLLMVLAYYRIYVTAKEHQQIQMLQRAGATSESRPQTADQHSTHR 250  
  
251 MRTETKAAKTLCVIMGCFCFCWAPFFVTNIVDPFIDYTVPEKWT AFLWL 300  
|||||  
251 MRTETKAAKTLCVIMGCFCFCWAPFFVTNIVDPFIDYTVPEKWT AFLWL 300  
  
301 GYINSGLNPFLY AFLNKSFRRAFLIILCCDDERYKRPPILGQTVPCSTTT 350  
|||||  
301 GYINSGLNPFLY AFLNKSFRRAFLIILCCDDERYKRPPILGQTVPCSTTT 350  
  
351 INGSTHVLRYTVLHSGQ.....HQELEKLPIHNDPESLES 385  
|||||| || :|| | .: : | .|| | :||  
351 INGSTHVLRDTVECGGQWESRCHLTATSPLVAAQPVIIRRPQDNLE..DS 398  
  
386 CF 387  
|  
399 CSLKRSQS 406

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**FIGURE 4-1**

**FIGURE 4-1.** 6/32  
**FIGURE 4-2.** 7/32  
**FIGURE 4-3.** 8/32

S10-95  
S10-87  
HP78  
S-H12 Rat  
S-H1C Rat  
His12 Dog

D L Y G H I L R S F L L P E V G R G L P D O L S P D O G G A D P V A G S W A P H L L S  
M E I L C E D N I S L S S I P N S L M W O L G O G P R L Y H N D F N S R  
W V N L G N A V R S L L M H L I G L

S10-95	N D R L D A N Y S S H E G F C S V E . . . . .	K Y V U L I F F A H 28
S10-87	H D R L D A N Y S S H E G F C S V E . . . . .	K Y V U L I F F A H 28
Hp78	E V T A S P A P I W D A P P D N A S G C G E Q I N Y Q R Y E . . . . .	K Y Y I G S I L T L 90
5-HT2 Rat	D A N T S E A S N W T I D A E N R T H L S C E G Y L P P T C L S I L H L Q E . . . . .	K N W S A L L I T V 83
5-HT1C Rat	L V W Q F D I S I S P V A A . . . . .	I V T D T F N S S D G G R L F Q F P D G V Q N W P A L S I V V 63
Hist2 Dog	H I S W G T G S S F C L D S P P C . . . . .	R I T V S V V L T V 27

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**FIGURE 4-2**

**FIGURE 4-3**

S10-95	GIVPCSTT	TINGSTHYL	- RDTYECGGQVE	SRCHELTATSPILVA	AQDPVIR	388
S10-87	GIVPCSTT	TINGSTHYL	- RYIYLHSSGH	DELEK	376	
HP78	LVNTIPALAYK	SVRNLNRYK	- SAGMHEALK	AERPERPEFVIA	NADYCRK	440
5-HT2 Rat	SVRNLNRYK	SVRNLNRYK	- KNSQEDAEQT	VDDCSMVTLGKA	QASEENCTD	459
5-HT1C Rat	SVRNLNRYK	SVRNLNRYK	- ALSGRELNVN	YARKANDPEPGI	EMAVENLELPVN	448
Hist2 Dog	SVRNLNRYK	SVRNLNRYK	- EISLRSSN	PHRQEELK	ARNASREPWSGTE	354

S10-95	R P Q D N D L E D G C S L K R S Q S	406
S10-87	L P I H N D P E S L E S C F	387
Hp78	K G H D S	445
5-HT2 Rat	N I E T V N E K V S S V	471
5-HT1C Rat	P S N V V S E R I S S V	460
Miss12 Dog	G A T Q R	359

FIGURE 5

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574	TTGGTCTATAAGAACAAAGATGACCCCTCTGGCATCGCATTAAATGCTGGGAGGCCGGCTGG	633
127	L V Y R N K M T P L R I A L W L G C C W	146
634	GTCATCCCCACGTTTATTCTTCTCCCTATAATGCAAGGCTGGAAATAACATTGGCATA	693
147	V I P T F I S F L P I M Q G W N N I G I	166
694	ATTGATTGTGATAGAAAAGAGGAAGGTTCAACCAGAAACTCTAACTCTACGTTACTGTGCTTC	753
167	I D L I E K R K F N Q N S N S T Y C V F	186
754	ATGGTCAACAAGCCCTAACGCCATCACCTGCTCTGGGGCCTTACATCCCATTCTC	813
187	M V N K P Y A I T C S V V A F Y I P F L	206
814	CTCATGGTGGCCTATTACCGCATCTATGTCACAGCTAACAGGCTAACGGCATGCCCATCAGATC	873
207	L M V L A Y Y R I Y V T A K E H A H Q I	226
874	CAGATGTTACAACGGGCAGGGCAGGAGCCCTCCGAGAGCAGGGCCTCAGTCGGCAGACCCGAT	933
227	Q M L Q R A G A S E S R P Q S A D Q H	246
934	AGCACTCATCCGATGAGGACAGGACAGAACGACAAAGGCCAAGACCCCTGTGCATCATGGGT	993
247	S T H P M R T E T K A A K T L C I I M G	266
994	TGCTTCTGCCCTCTGGCACCATTCCTTGTCAACATTGTTGGATCCTTCATAGAC	1053
267	C F C L C W A P F F V T N I V D P F I D	286
1054	TACACTGTCCCTGGCAGGTGTGACTGGCTTTCTGGCTGGCTATATCAATTTC	1109
287	Y T V P G Q V W T A F L W L G Y I N	304

FIGURE 6

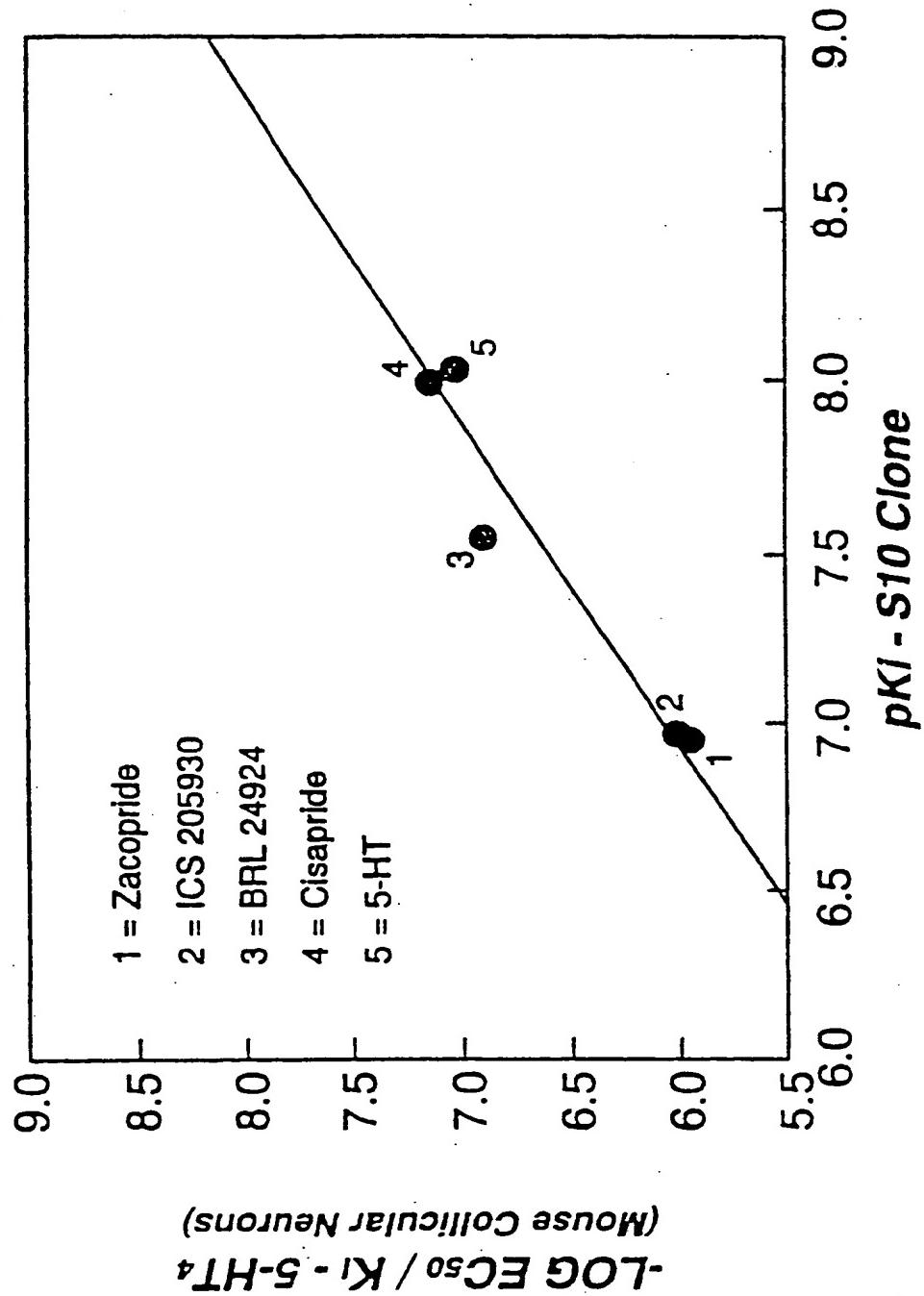
574	TTGGTCTATAGGAACAGATGACCCCTCTGGCATCGCATTAAATGCTGGGGCTGCTGC	633
	T A	A
634	GTCATCCCCACGTTTATTTCCTTCTCCCTATAATGCAAGGCTGGAAATAACATTGGCATA	693
	T T A	C C
694	ATGATTGATAGAAAAGGAAAGTTAACCGAGACTCTAACCTACGTACTGCTCTTC	753
G G	A A	C A T
754	ATGGTCAACAAAGCCCTAACGCCATCACCTGCTCTGTGCTTACATCCCATTCTCTC	813
	T	G
814	CTCATGGTGGCTATTACGGCATCTATGTCACAGGCTAAGGAGCATGCCATCAGATC	873
	T	T
874	CAGATGTTACAACGGGCAAGGAGCCTCCGAGAGGAGCCCTCAGTGGCAGACCGCAT	933
	A T A	C A A T
934	AGCACTCATCCGATGAGGACAGAGACCAAGCAGCCAAAGCACCCCTGTGCATCATGGGT	993
A A	GC C	TT A TG
994	TGCTTCTCCCTGCTGGCACCATTCCTGTCACCAATAATTGTGATCCCTTCATAGAC	1053
	TT C C	C
1054	TACACTGTCCTGGCACGGTGGACTGCTCCCTGCTGGCTATATCAATTTC	1109
	G C A A	T

FIGURE 7

127 L V Y R N K M T P I R I A L M L G G C W V I P T F I S F L P I M Q G W N N I G I I D L I E K R K F N Q N S N S T Y C V F 186  
M V V H F  
187 M V N K P Y A I T C S V V A F Y I P F L L M V L A Y Y R I Y V T A K E H A H Q I Q M L Q R A G A S S E S R P Q S A D Q H 246  
Q T T  
247 S T H P M R T E T K A A K T L C I I M G C F C L C W A P F F V T N I V D P F I D Y T V P G Q Q V W T A F L W L G Y I N 304  
R V F E K

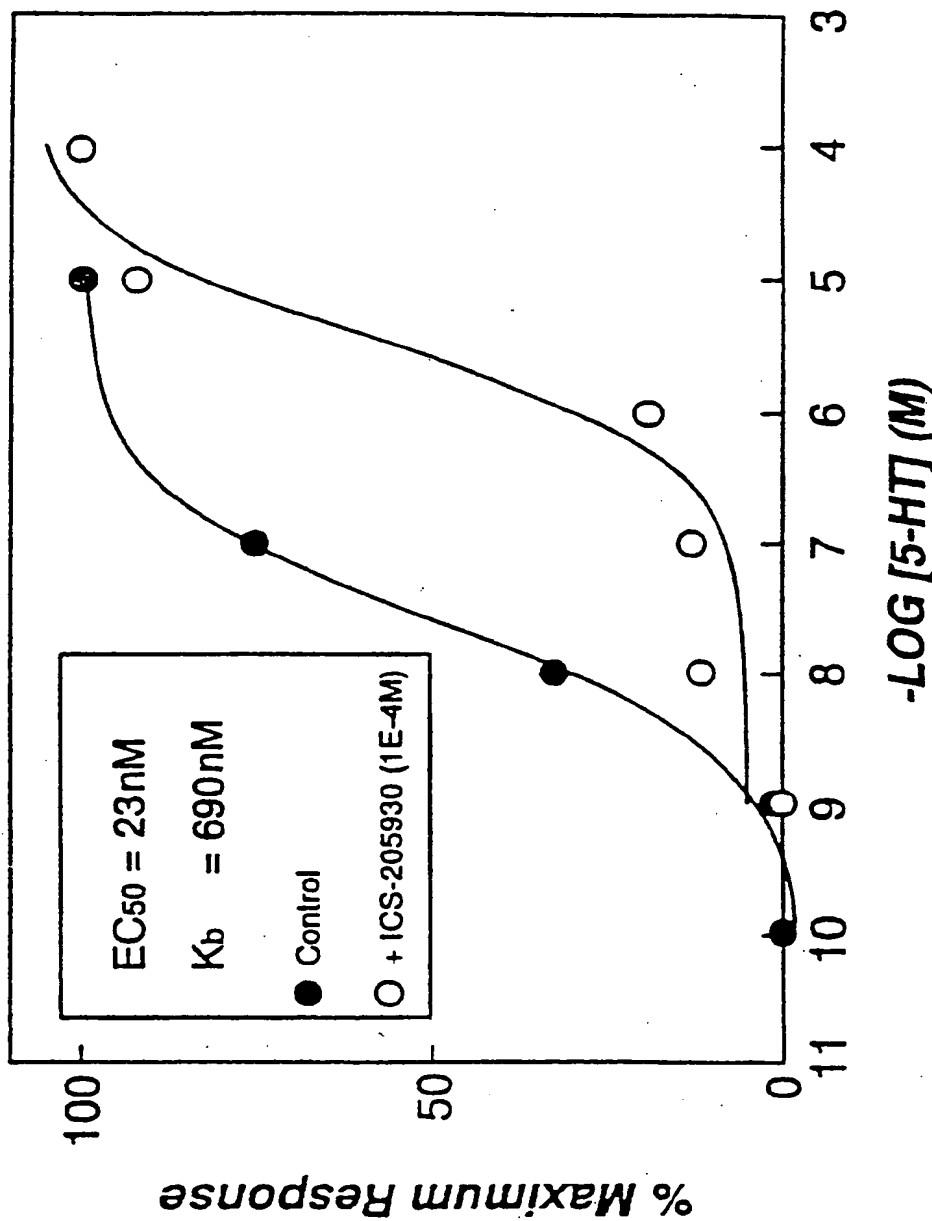
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FIGURE 8



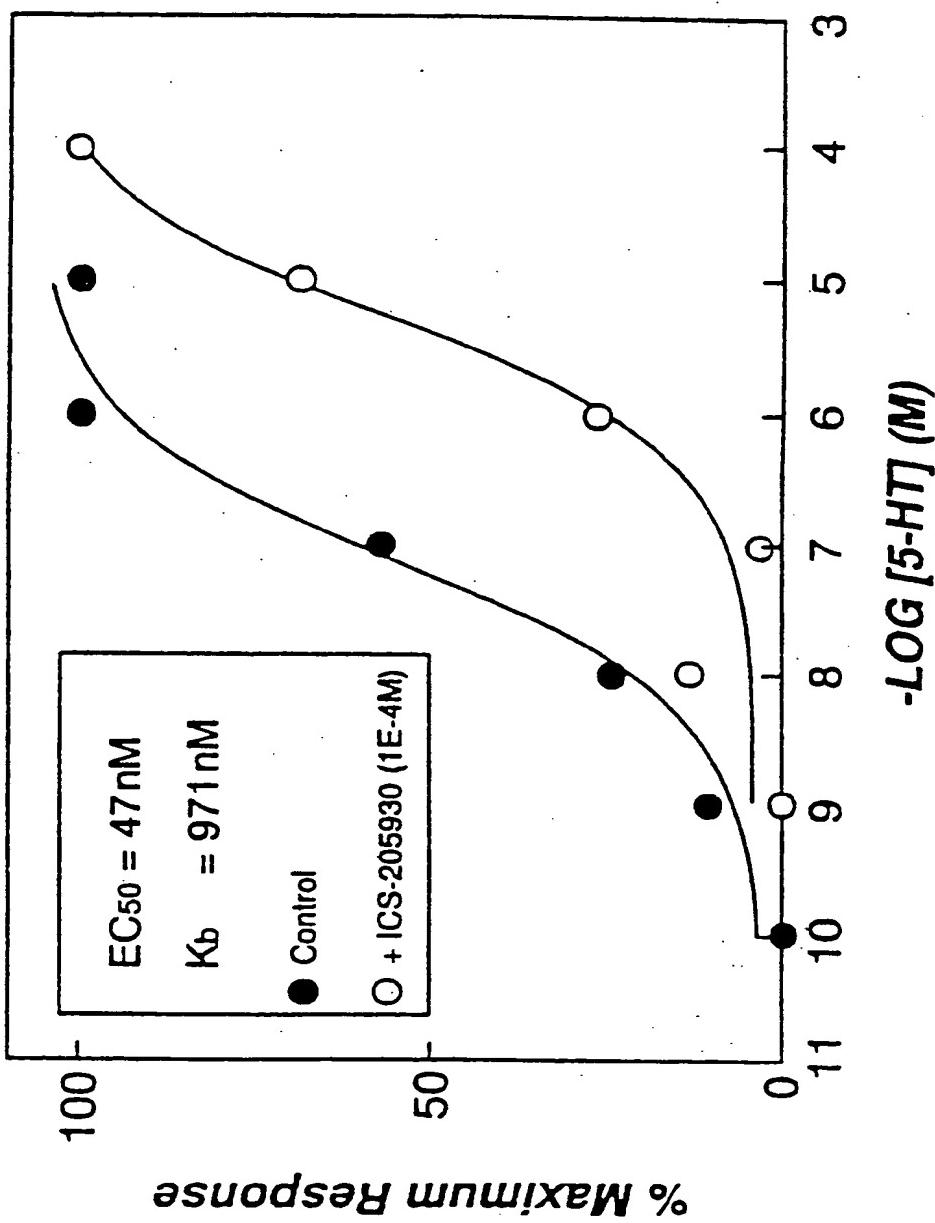
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FIGURE 9



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FIGURE 10



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**FIGURE 11A**

1 TTGGCTATAAGAACAAAGATGACCCCTCTGGCATTAATGGCTGGGAGGCTGGTGG  
 61 GTCATCCCCACGTTATTCTTCTCCCTATAATGCAAGGCTGGAAATAACATTGGCATA  
 121 ATGGATTGATAGAAAAGAGGAAGTTCAACCAGAAACTCTAACTCTACGGTACTGTGTC  
 181 ATGGTCAACAAGGCCCTACGCCATCACCTGCTCTGTGGTGCCTCTACATCCCATTCTC  
 241 CTCATGGTGCCTGGCTTACCGCATCTACAGCTATGTCACAGCTAAGGAGCATGCCATTGAGTC  
 301 CAGATGTTACAACGGGCAGGGCCTCCGGAGGAGCCAAAGCAGGCCAACAGGAGACCA  
 361 AGCACTCATGGCATGGACAGAGACAGAGACAGAGACAGAGACAGAGACAGAGACCA  
 421 TGCTTCTGGCCTCTGCTGGCACCATTTGTCAACCAATTGGATCCTGGCTATATCAATT  
 481 TACACTGTCCCTGGCAGGGTGTGGACTTGCTTCTGGCTGGCTATGGCTGGCTGG  
 541 TTGAAACCCCTTTCTACGGCTTCTGAAATAAGTCTTGTAGACGTGGCTGGCTGG  
 601 CTCTGCTGTGATGATGAGGCTACCGAAGACCTCCATTCTGGGCCAGACTGTCCCTGT  
 661 TCAACCACACCATTAATGGATCCACACATGTACTAACACATGGAAACTGCCATAC  
 721 CATCATCAGGAACACTCGAGAACAAACTGGCCATACACAAATGGAAATCCCTGGAA  
 781 TTCTGATTGAGG 792

**FIGURE 11B**

1 R I A L M Q N S V T A K P R Q E S A D H Q M I D G C W  
 21 P I M N Q S V T A K P R Q E S A D H Q M I D G C W  
 41 F L K I R Y A Y G A T E P F T A K V I D P N S I  
 61 N K I F E K P A Y R M R C W Q P F T A K V I D P N S I  
 81 Y P T F L K V L Q R M C W Q P F T A K V I D P N S I  
 101 R N K I F E K P A Y R M R C W Q P F T A K V I D P N S I  
 121 L Q S T F C V P L G Y A R G S T E N G L E K  
 141 C M M T H C V P L G Y A R G S T E N G L E K  
 161 Y L N P F L D E N G L E K  
 181 L C T Q P D T I E H \*  
 201 S T H F D T I E H \*  
 221 S H F D T I E H \*  
 261 F D T I E H \*

20 40 60 80 100 120 140 160 180 200 220 240 260

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FIGURE 12-1, 16/32
FIGURE 12-2, 17/32
FIGURE 12-3, 18/32

**FIGURE 12-1**

1                   TTGGTCTATGGAACAAAGATGA 22  
 451 TAGGTATTATGCCATCTGCTAACCTTGTTATAGAAACAAAGATGA 500

23 CCCCTCTGGCATCGCATTAATGCTGGGCTGCTGGTCATCCCCACG 72  
 501 CCCCTCACGGCATCGCATTAATGCTGGAGGCTGGTCATTCCCATG 550

73 TTTATTCCTTTCCTCCATAATGCAAGGGCTGGAAATAACATGGCATATAAT 122  
 551 TTTATCTTCTCCATAATGCAAGGGCTGGAAACACATGGGCATAGT 600

123 TGATTTGATAGAAAAGAGGAAGTCAACCGAAACTCTAACTCTACGTACT 172  
 601 TGATGTGATAGAGAAAAGGAATTCACCAACTCTAACTCTACATCT 650

173 GTGTCCTCATGGTCAACAAAGCCCTACGCCATCACCCCTGCTCTGTGGGCC 222  
 651 GTGTCCTCATGGTCAACAAAGCCCTATGCCATCACCCCTGCTCTGTGGGCC 700

223 TTCTACATCCATTTCTCATGGTGGCCTATTACCGCATCTATGT 272  
 701 TTCTACATCCGTTCTCATGGCTGGCCATTACCGTATCTATGT 750

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**FIGURE 12-2**

273	CACAGCTAAGGAGCATGCCATCAGATCCAGATTTACAACGGGCAGGAG	322
751	CACTGCTAAGGAGCATGCCAGCAGATCCAGATTTACAACGGGCAGGAG	800
323	CCTCCCTCCGAGGAGCCAGGGCCTCAGTCGGCAGACCAGCATAGCACCTCATCGC	372
801	CCACCTCTGAAGCAGGCCAGACAGCTGACCAGCACAGCACACATCGC	850
373	ATGAGGACAGACCAAAAGCAGCCAAAGACCCCTGTGCATCATGGGTG	422
851	ATGGGGACAGACCAAAAGCAGCCAAAGACCTTATGTGTCAATCATGGGCTG	900
423	CTTCTGCCTCTGCTGGCACCATTCCTTGTCACCAATAATTGTGGATCCTT	472
901	CTTCTGTTCTGCTGGCCCCCTCTTGTCACCAATAATTGTGGACCCCTT	950
473	TCATAGACTACACTGTCCCTGGCAGGTGTGGACTGCTTCCCTCTGGCTC	522
951	TCATAGACTACACTGTGCCCGAGAAGGTGTGGACTGCTTCCCTCTGGCTT	1000
523	GGCTATATCAATTCCGGGTGAACCCCTTCTCTACGCCCTCTTGAAATAA	572
1001	GGCTATATCAATTCAAGGGTTGAACCCCTTCTCTATGCCCTCTTGAAATAA	1050

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FIGURE 12-3

573	GTCTTTAGACGTCGCCTCCTCATCATTCTGCTGTGATGATGAGGGCT	622
1051	GTCTTTCAGACGTCGCCTTATCATCCTCTGCTGTGATGATGAGGGCT	1100
623	ACCGAAGACCTCCATTCTGGCCAGACTGTCCCTGTCAACCACAAACC	672
1101	ACAAAAGACCCCCATTCTGGCCAGACTGTCCCTGTCAACCACAAACC	1150
673	ATTAATGGATCCACACATGTAAGGTACACCGTTCTGCACACGGGACA	722
1151	ATTAATGGATCCACCTCATGGTATAACAGTTTGCAATAGTGGTCA	1200
723	TCATCAGGAACCTGAGAAACTGCCATACACAAATGACCCAGAACCCCTGG	772
1201	ACACAGGAACCTGGAGAAGTTGCCATACACAAATGACCCAGAGTCCCTGG	1250
773	AATCATGCTTCTGATTGAGG.....	792
1251	AATCATGCTTGTGAGACGGTGGCTTAGGGTTAGCCATCCCCAT	1300

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**FIGURE 13**

1		L V Y R N K M T P L R I A L M L G G C W V I P T	24
101	V L L T A S I F H L C C I S I L D R Y Y A I C C Q B L	V Y R N K M T P L R I A L M L G G C W V I P M	150
25	F I S F L P I M Q G W N N I G I I D L I E K R K F N Q N S N S T Y C V F M V N K P Y A I T C S V V A		74
151	F I S F L P I M Q G W N N I G I V D V I E K R K F N H N S N S T F C V F M V N K P Y A I T C S V V A		200
75	F Y I P F L I M V L A Y Y R I Y V T A K E H A H Q I Q Q M L Q R A G A S S E S R P Q S A D Q H S T H R		124
201	F Y I P F L I M V L A Y Y R I Y V T A K E H A Q Q I Q Q M L Q R A G A T S E S R P Q T A D Q H S T H R		250
125	M R T E T K A A K T L C I I M G C F C L C W A P F F V T N I V D P F I D Y T V P G Q V W T A F L W L		174
251	M R T E T K A A K T L C V I M G C F C F C W A P F F V T N I V D P F I D Y T V P E K V W T A F L W L		300
175	G Y I N S G L N P F L Y A F L N K S F R R A F L I I L C C D D E Y R R P S T I L G Q T V P C S T T T		224
301	G Y I N S G L N P F L Y A F L N K S F R R A F L I I L C C D D E Y K R P P I L G Q T V P C S T T T		350
225	I N G S T H V L R Y T V L H R G H H Q E L E K L P I H N D P E S L E S C F *		262
351	I N G S T H V L R Y T V L H S G H H Q E L E K L P I H N D P E S L E S C F *		387

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FIGURE 14A

FIGURE 14A, 20/32
FIGURE 14B, 21/32

CCTGTAATGGACAAACTTGATGCTTAATGAGTTCTGAGGTCTGAGGGGTTTCAGTGGAG  
 1 61 AAGGTGGTGGCTGCTCACGGTTCTCGACGGTTATCCTGATGGCCATCTTGGGAACCTG  
 1.21 CTGGTGATGGTGGCTGTTGCTGGATCTGGCTGGTTTCGGGAGCTCAGGAAATAAAACAAATTATTC  
 181 ATTGATCTCTGGCTTCAAGACATCTGGATTATGGGAGGTGTTTGTCTTGGACATCT  
 241 ATTGAGCTGGTCAAGACATCTGGATTATGGGAGGTGTTTGTCTTGGACATCT  
 301 CTGGACGGTCTGGCTCACAAACGGCATCGATTTCACCTGTGCATTCTGGATAGG  
 361 TATTACGCCATCTGCTGCCAGCCTTGGCTCATCCCCACGTTATTAGGAACAAGATGAC  
 421 GCATTAATGCTGGAGGGCTGCTGGGTCTATGGCTTATAGAACAGGAAAGTCAACCAGAAC  
 481 CAAGGCTGGAAATAACATGGCATTAATTGATTGATAGAAAAGGAAGGAAAGTCAACCAGAAC  
 541 TCTAACTCTACGTACTGTGTTCATGGTCAACAAAGCCCTAGGCCATCACCTGCTCTGTG  
 601 GTGGCCCTTACATCCCATTCTCCTCATGGTGGCTTATACCGCATCTATGTCA  
 661 GCTAAGGAGCATGCCATCAGATCCAGATGGTACAAACGGGAGGGCCTCCGAGAGC  
 721 AGGCCTCAGTGGCAGACCAGCATAGGCACTCATGGCATAGGACAGAACAGGCC  
 781 AAGACCCCTGGCATCATGGTTGGCTCTGGCCTCTGGCACCATTCTTGTCA  
 841 AATATTGTGGATCCCTCATAGACTACACTGTCCCTGGCAGGGTGGACTGCTCT  
 901 TGGCTCGGCTATATCAATTCCGGTTGAACCCATTACGGCTTCTTGAATAAGTCT  
 961 TTAGACGGTGGCTTCCCTCATCATCCTCTGGTGTGAGTGAAGGGCTACCGAACCTCC  
 1021 ATTCGGCCAGACTGTCCCTGGTCAACCACCATTAATGGATCCACACATGTACTA  
 1081 AGGGATGCCAGTGGAGTGGTGGCCAGTGGAGGTCAAGTGTGCAGTGTCA  
 1140 CCTTTGGCTGGCTCAGCCCCAGTGAACACTTAGGCCCTGGACAATGACCCAGAGACA  
 1200 GCCATGGCTCCGAAGAGGGCCAGGTCCTAAGCTGGCTGCTGGCTGGCTGG  
 1260 ATTCTCTTACCTGAGGCTTCCGGTCCAGTGCAGGAACCCGGTGGCTGG  
 1.261

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FIGURE 14B

	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320	340	360	380	400
1	21	41	61	81	101	121	141	161	181	201	221	241	261	281	301	321	341	361	381	
	E	L	F	A	S	R	I	M	N	V	T	S	A	T	L	S	S	L	S	
	V	N	Y	G	T	D	R	I	Q	S	V	E	A	V	F	K	P	V	T	
	S	G	N	F	R	L	L	P	N	C	Y	S	K	F	A	N	R	H	A	
	G	L	T	P	V	S	P	L	F	T	I	S	T	F	T	L	R	T	P	
	F	I	K	M	L	I	T	F	K	I	R	A	E	P	W	F	Y	S	P	
	G	A	I	V	C	C	M	S	R	A	Y	G	T	A	V	A	R	G	H	
	E	M	K	L	F	C	K	I	K	Y	Y	A	R	W	Q	Y	E	N	C	
	E	L	R	V	V	L	N	F	B	P	A	R	M	C	G	L	D	I	Q	
	S	I	L	S	E	H	R	T	I	K	L	Q	R	L	P	F	D	T	S	
	S	V	Q	V	G	F	Y	P	L	N	V	L	H	C	V	P	C	T	E	*
	V	T	R	L	Y	I	V	I	D	V	M	M	T	F	T	N	C	T	W	T
	N	S	D	L	I	S	L	V	I	M	L	Q	S	C	Y	L	S	Q	D	
	A	L	W	D	W	A	P	W	I	F	L	I	H	G	D	G	I	C	G	S
	D	F	C	A	I	T	Q	C	G	V	F	Q	Q	M	I	S	I	P	G	P
	L	T	V	F	D	T	C	G	I	C	P	H	D	I	F	N	L	V	C	Q
	K	L	A	A	Q	L	C	G	N	Y	I	A	A	I	P	I	F	T	E	A
	D	L	V	L	V	L	I	L	N	T	Y	H	S	C	D	Y	A	Q	V	A
	M	V	M	S	L	V	A	M	W	S	F	E	Q	L	V	G	R	G	A	V
	V	V	V	E	D	Y	L	G	N	A	K	P	T	I	L	R	L	D	L	
	K	L	I	I	L	Y	A	Q	S	V	A	R	N	F	I	I	R	P	P	

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**FIGURE 15-1**

FIGURE 15-1, 22/32
FIGURE 15-2, 23/32
FIGURE 15-3, 24/32
FIGURE 15-4, 25/32

7 ATGGACAAACTTGATGCTAATGTGAGTTCTGAGGAGGGTTTCGGTCACT 56  
 152 ATGGACAGAGCTTGATGCTAATGTGAGTTCAACGAGGGTTCCGGTCTGT 201

57 GGAGAAGGGTGGTCTGCTCACGTTTCTCGACGGTTATCCTGATGGCCA 106  
 202 GGAGAAGGGTACTGCTCACGTTCTCGCAATGGTTATCCTGATGGCCA 251

107 TCTTGGGAACCTGGTGTGATGGCTGTGGCTGCTGGACAGGCCAGCTC 156  
 252 TCC'GGCAACCTGCTGGTGTGATGGCTGTGGCAGGGACAGGCAGCTC 301

157 AGGAAAATAAAACAAATTTCATTGATCTCTTGGATCTTGGGATCT 206  
 302 AGGAAAATAAAACCAATTTCATTGATCTCTTGGCTTGCTGATCT 351

207 GCTGGTTTGGTGTGGTGTGGCTGGCATTGGCTGGTCAAG 256  
 352 GCTGGTTTGGTGTGGTGAATGCCCTGGCATGGTCAAG 401

257 ACATCTGGATTATGGGAGGGTGTGTTGCTGGACATCTCTGGAC 306  
 402 ACATCTGGTTTATGGGagatttggccctggacacctctggat 451

307 GTCCTGCTCACAACGGCATTGATTTCACTGCTGCAATTCTCTGGGA 356  
 452 gtcctactaccacagcatcaattttcacctctgctgcatttccctGGGA 501

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FIGURE 15-2

357	TAGGTATTACGCCATCTGCTGCCAGCCTTGGTCTATAGAACAAAGATGA	406
502	TAGGTATTATGCCATCTGCTGTCAACCTTGGTTATAGAACAAAGATGA	551
407	CCCCCTCTGGCATGCCATTAAATGCTGGAGGGCTGGGTCAATCCCCACCG	456
552	CCCCCTCTAGGCATGCCATTAAATGCTGGAGGGCTGGGTCAATCCCCATG	601
457	TITATTTCTTTCTCCCTATAATGCAAGGGCTGGAAATAACATGGCATAAT	506
602	TATCTTTCTCCCTATAATGCAAGGGCTGGAAACAACATGGCATAAGT	651
507	TGATTGATAGAAAAGAGGGAAACTCAACCAACTCTAACTCTACGTACT	556
652	TGATGTGATAGAGAAAAGGAAATTCAACCACAAACTCTAACTCTACATTCT	701
557	GTGTCTTCATGGTCAAACAGCCCTACGCCATCACCTGCTCTGGTGGCC	606
702	GTGTCTTCATGGTCAAACAGCCCTATGCCATCACCTGCTCTGGTGGCC	751
607	TCTACATCCCATTTCTCCCTCATGGTGGCTTATTACCGCATTCTATG	656
752	TTCTACATCCCGTTCTCCCTCATGGTGGCTTATTACCGTATCTATG	801

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FIGURE 15-3

657	CACAGCTAAGGAGCATGCCATCAGATCCAGATGTTACAACGGGCAGGAG	706
802	CACTGCTAAGGAGCATGCCAGCAGATCCAGATGTTACAACGGGCAGGAG	851
707	CCTCCTCGAGGAGGCCCTCAGTCGGCAGCAGCATAGCACTCATCGC	756
852	CCACCTCTGAAAGGAGCCCCAGACAGGTGACCAGCACACATCGC	901
757	ATGAGGACAGAGACCAAAGCAGGCCAAGAACCCCTGTGCATCATGGGTG	806
902	ATGGGGACAGAGACCAAAGCAGGCCAAGAACCTTATGTGTCAATCATGGGCTG	951
807	CTTCCTGCCTCTGCTGGCACCATTCCTTGTCACCAATAATTGTGGATCCTT	856
952	CTTCCTGTTCTGCTGGCCCCCTCTTTGTCACCAATAATTGTGGACCCCTT	1001
857	TCATAGACTACACTGTCCCCGGCAGGGTGTGGACTGCTTCTGGCTC	906
1002	TCATAGACTACACTGTGCCGGAGAAGGTGTGGACTGCTTCCCTCTGGCTT	1051
907	GGCTATCAATTCCGGGTGAACCCCTTCTACGCCCTCTTGAAATAA	956
1052	GGCTATCAATTCAAGGGTTGAACCCCTTCTATGCCCTCTTGAAATAA	1101

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FIGURE 15-4

957 GTCTTTAGACGTGCCCTCCTCATCATCCCTCTGCTGTGATGAGGGCT 1006  
1102 GTCTTCAGACGTGCCCTCCTTATCATCCCTCTGCTGTGATGAGGGCT 1151

1007 ACCGAAGACCTCCATTCTGGCCAGACTGTCCCTTGTCAACCACAAACC 1056  
1152 ACAAAAGACCCCCATTCTGGCCAGACTGTCCCTGTCAACCACAAACC 1201

1057 ATTAATGGATCCACACATGTTACTAAGGGATGGCAGTGGACTGTGGCCA 1106  
1202 ATTAATGGATCCACCTCATGGCTAAGGGATAACAGTGGAAATGTGGCCA 1251

1107 GTGGGAGAGTCAGTGTCAACCGCCAGCAACTTCTCCTTGTGGCTGCTC 1156  
1252 ATGGGAGAGTCGGTGTCAACCTCACAGCAACTTCTCCTTGTGGCTGCTC 1301

1157 AGCCCCAGTGACACTTAG..... 1173  
1302 AG.CCAGTGATACTGTAGGGCCCCAGGACAATGACCTAGAAGACAGCTGTAG 1350

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FIGURE 16-1

FIGURE 16-1, 26/32

FIGURE 16-2, 27/32

1 MDKLDANVSSSEEGFGSVEKVVLITFLSTVILMAILGNILLVMVAVCWDRQL 50  
1 MDRILDANVSSNEGFGSVEKVVLITFFAMVILMAILGNILLVMVAVCRDRQL 50

51 RKIKTNYFIVSLAFADLLVSVLVMPFGAIELVQDIWIYGEVFCLVRTSLD 100  
51 RKIKTNYFIVSLAFADLLVSVLVNAFGAIELVQDIWFYGYEMFCLVRTSLD 100

101 VLLTTASIFHLCCISLDRYYAICCQPLVYRNKMTPLRIALMLGGCVV IPT 150  
101 VLLTTASIFHLCCISLDRYYAICCQPLVYRNKMTPLRIALMLGGCVV IPM 150

151 FISFLPIMQGWNNIGIIDLIEKRKFENQNSNSTYCVFMVNKPYAITCSVVA 200  
151 FISFLPIMQGWNNIGIVDIEKRKFHNNSNSTFCVFMVNKPYAITCSVVA 200

201 FYIPFLLMVLAYRIYVTAKEHQIQMLQRAGASSESRPQSADQHSTHR 250  
201 FYIPFLLMVLAYRIYVTAKEHQQIQMQLQRAGATSESRPQTADQHSTHR 250

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## FIGURE 16-2

251 MRTETKAAKTLLCIMGFCCLCWAPFFVVTNIVDPEIDYTVPGQVWTAFLWL 300  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
251 MRTETKAAKTLLCVIMGFCFCWAPFFVVTNIVDPEIDYTVPEKUWTAFLWL 300  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
301 GYINSGLNPNPFLYAFLINKSFRRAFLLILCCDDERYRRPSIILGQTVPSCSTTT 350  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
301 GYINSGLNPNPFLYAFLINKSFRRAFLLILCCDDERYKRPPILGQTVPSCSTTT 350  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
351 INGSTHVLRDAVECGGQWESQCHPPATSPLVAAQPSDT\* ..... 389  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
351 INGSTHVLRDTVECGGQWESRCHLTATSPLVAAQPVIRRPQDNLEDSCS 400  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
401 LKRSQS\* ..... 406  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**FIGURE 17-1**

FIGURE 17-1, 28/32
FIGURE 17-2, 29/32
FIGURE 17-3, 30/32

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357 TAGGTATTACGCCATCTGCTGCCAGCCTTGGCTATAAGAACAAAGATGA 406  
 1 ..... TTGGTCTATAAGAACAAAGATGA 22

407 CCCCTCTGGCATCGCATTAAATGCTGGAGGCTGCTGGTCATCCCCACG 456  
 23 CCCCTCTGGCATCGCATTAAATGCTGGAGGCTGCTGGTCATCCCCACG 72

457 TTTTATTCTTTCTCCCTATAATGCCAAGGCCTGGAAATAACATTGGCATAAT 506  
 73 TTTTATTCTTTCTCCCTATAATGCCAAGGCCTGGAAATAACATTGGCATAAT 122

507 TGATTGTGATAGAAAAGAGGAAGTTCAACCAAGAACACTCTAACTCTACGTACT 556  
 123 TGATTGTGATAGAAAAGAGGAAGTTCAACCAAGAACACTCTAACTCTACGTACT 172

557 GTGCTCTCATGGTCACACAGCCCTACGCCATCACCTGCCCTGTGGTGGCC 606  
 173 GTGCTCTCATGGTCACACAGCCCTACGCCATCACCTGCCCTGTGGTGGCC 222

607 TTCTACATCCCATTCTCCTCATGGCTGGCCTTACCGCATTACCGCATCTATGT 656  
 223 TTCTACATCCCATTCTCCTCATGGCTGGCCTTACCGCATTACCGCATCTATGT 272

657 CACAGCTAAGGAGGATGCCATCAGATCCAGATGGCTTACAACGGGCAGGAG 706  
 273 CACAGCTAAGGAGGATGCCATCAGATCCAGATGGCTTACAACGGGCAGGAG 322

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FIGURE 17-2

707	CCTCCTCCGAGCAGGGCCTCAGTCGGCAGACCGATAGCACTCATCGC	756
323	CCTCCTCCGAGCAGGGCCTCAGTCGGCAGACCGATAGCACTCATCGC	372
757	ATGAGGACAGAGACCAAAGGCCAAGCAGCAGCCAAAGGCAAGCACCCTGTGCATCATGGTTG	806
373	ATGAGGACAGAGACCAAAGGCCAAGCAGCAGCCAAAGGCAAGCACCCTGTGCATCATGGTTG	422
807	CTTCTGCCTCTGCTGGCACCAATTCTTGTCAACCAATTGTGGATCCTT	856
423	CTTCTGCCTCTGCTGGCACCAATTCTTGTCAACCAATTGTGGATCCTT	472
857	TCATAGACTACACTGTCCCCTGGCAGGGTGTGGACTGCTTCTGGCTC	906
473	TCATAGACTACACTGTCCCCTGGCAGGGTGTGGACTGCTTCTGGCTC	522
907	GGCTATAATCAATTCCGGGTTGAACCCCTTCTCTACGCCCTCTTGAAATAA	956
523	GGCTATAATCAATTCCGGGTTGAACCCCTTCTCTACGCCCTCTTGAAATAA	572
957	GTCTTTAGACGGCCCTCATCATCCTCTGCTGTGATGAGGGCT	1006
573	GTCTTTAGACGGCCCTCATCATCCTCTGCTGTGATGAGGGCT	622

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FIGURE 17-3

1007 ACCGAAGACCTTCCATTCTGGCCAGACTGTCCTGTTCAACCAACC 1056  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
623 ACCGAAGACCTTCCATTCTGGCCAGACTGTCCTGTTCAACCAACC 672  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
1057 ATTAATGGATCCACACATGTAAGGGATGGCAGTGGAGTGTGGCCA 1106  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
673 ATTAATGGATCCACACATGTAAGGTACACCGTTCTGGCACAGGGACA 722  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
1107 GTGGGAGAGTCAGTGTCACCCCAGCAACTTCTCCTTGGCTGCTC 1156  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
723 TCATCAGGAACCTGGAGAAACTGCCATACACAATGACCCAGAATCCCTGG 772  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
1157 AGCCAGTGACACTTAG 1173  
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |  
773 AATCATGCTTCTGA... 786

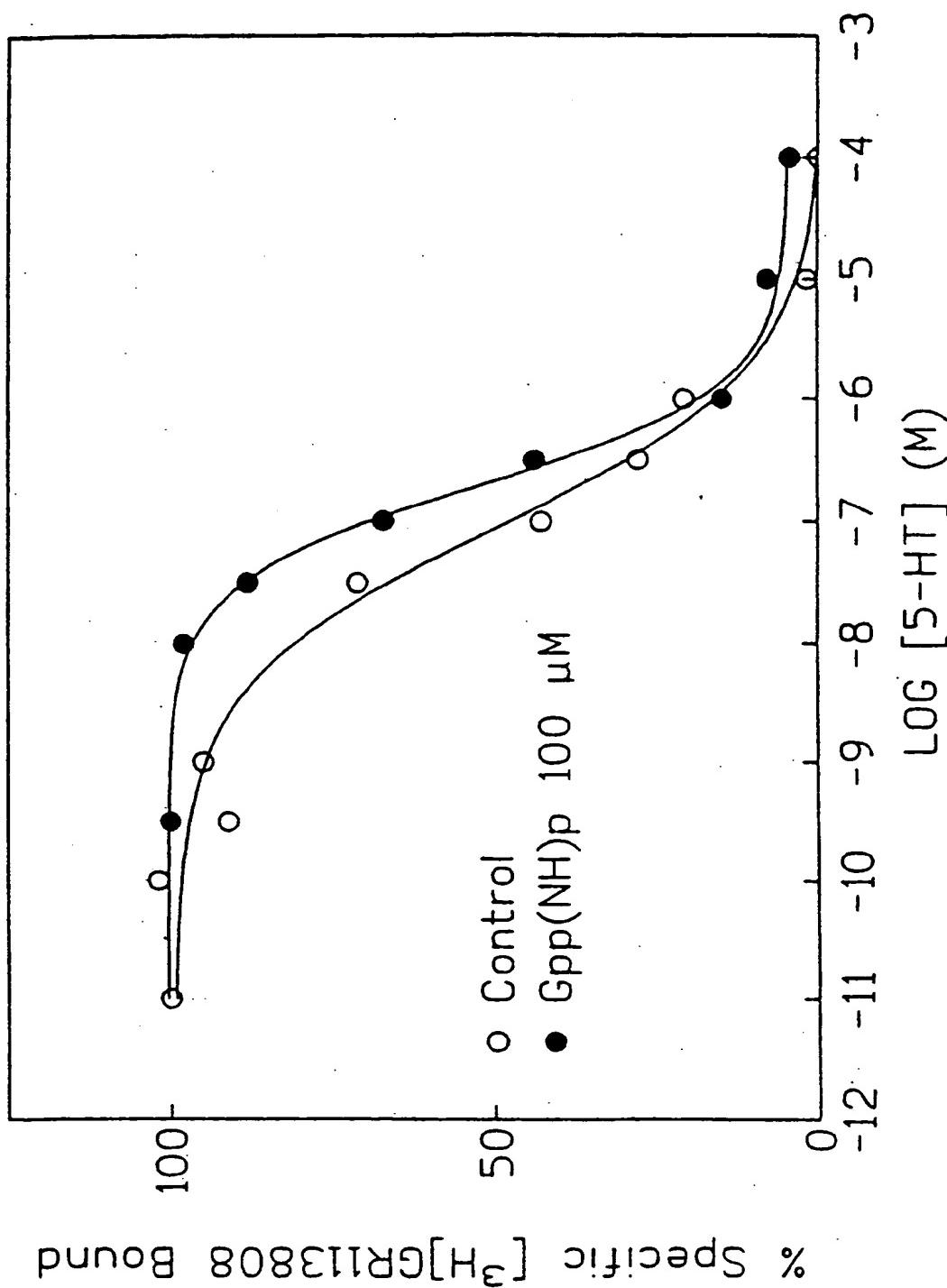
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**FIGURE 18**

101	VLLTTASIFHLCCISLDRYYAICCQPLVYRNMKTPLRIALMLGGCWVIPT	150
1	.....L.....	.....L.....
151	FISFLPIMQGWNНИGІІDЛIEKRKFНQNNSNSTYCVFMVNKPYAITCSVVA	200
25	FISFLPIMQGWNНИGІІDЛIEKRKFНQNNSNSTYCVFMVNKPYAITCSVVA	74
201	FYIPFLLMVLAYRIYVTAKEHАHQIQMЛQRAGASSESRPQSADQHSTHR	250
75	FYIPFLLMVLAYRIYVTAKEHАHQIQMЛQRAGASSESRPQSADQHSTHR	124
251	MRTETKAAKTLCIIMGCFCLCWAPFFVTNIVDPFIDYTVPGQQVWTAFLWL	300
125	MRTETKAAKTLCIIMGCFCLCWAPFFVTNIVDPFIDYTVPGQQVWTAFLWL	174
301	GYINSGLNPFLYAFLNKSFRRAFLIIЛCCDDERYRRPSILGQTVPCSTTT	350
175	GYINSGLNPFLYAFLNKSFRRAFLIIЛCCDDERYRRPSILGQTVPCSTTT	224
351	INGSTHVLRLDAVECGGQWESQCHPPATSPLVAAQPSDT*	389
225	INGSTHVLRLYTVLHRGHHQELEKLPIHNDPESLESCF*	262

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FIGURE 19



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/12586

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C12N 1/15, 1/21, 5/10, 15/00, 15/12

US CL : 435/6, 172.3, 240.2, 252.3, 255.1, 320.1; 536/23.5, 24.3, 24.31

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 172.3, 240.2, 252.3, 255.1, 320.1; 536/23.5, 24.3, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (files 5, 155, 351, 357, 358) search terms: serotonin, receptor, DNA, 5-HT

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,985,352 (JULIUS ET AL) 15 January 1991, see Figure 1.	25-28
A, P	US, A, 5,242,822 (MARULLO ET AL) 07 September 1993, see abstract and claims.	1-37, 40-42
A	FEBS, Volume 312, Number 2,3, issued November 1992, Loric et al, "New mouse 5-HT2-like receptor", pages 203-207, see entire document.	1-37 and 40-42

 Further documents are listed in the continuation of Box C. See patent family annex.

*A*	Special categories of cited documents:	"T"	later documents published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)	"&"	document member of the same patent family
"P"	document referring to an oral disclosure, use, exhibition or other means		
	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

07 June 1994

Date of mailing of the international search report

JUN 20 1994

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/12586

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims N.s.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-37 and 40-42

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as follows:

- I. Claims 1-37 and 40-42, drawn to nucleic acid sequences for the 5-HT<sub>4</sub> receptor, vectors, host cells, probes, antisense nucleic acids, and a method for detecting expression of a mammalian 5-HT<sub>4</sub> receptor, classified in at least, for example, Class 536, subclass 23.5.
- II. Claims 38-39, drawn to a method of isolating a gene, classified in at least, for example, Class 435, subclass 172.3.
- III. Claims 43-44, drawn to a 5-HT<sub>4</sub> receptor protein, classified in at least, for example, Class 530, subclass 350.
- IV. Claims 45-48, drawn to a method of production of a mammalian 5-HT<sub>4</sub> receptor, classified in at least, for example, Class 435, subclass 69.1.
- V. Claims 49-54 and 61, drawn to an antibody to a 5-HT<sub>4</sub> receptor protein, classified in at least, for example, Class 530, subclass 387.1.
- VI. Claim 55, drawn to a pharmaceutical composition to alleviate abnormalities resulting from overexpression of a 5-HT<sub>4</sub> receptor, classified in at least, for example, Class 514, subclass 2.
- VII. Claim 56, drawn to a pharmaceutical composition to alleviate abnormalities resulting from underexpression of a 5-HT<sub>4</sub> receptor, classified in at least, for example, Class 514, subclass 2.
- VIII. Claims 57-60, drawn to pharmaceutical compositions containing oligonucleotides, classified in at least, for example, Class 514, subclass 44.
- IX. Claims 62-66, drawn to transgenic animals, classified in at least, for example, Class 800, subclass 2.
- X. Claims 67-68, drawn to a method for determining physiological effects of varying levels of 5-HT<sub>4</sub> receptors using transgenic animals, classified in at least, for example, Class 514, subclass 44.
- XI. Claims 69-70, drawn to a method for determining specific binding using whole cells, classified in at least, for example, Class 435, subclass 7.2.
- XII. Claims 71-72, drawn to a method of screening compounds to identify drugs using whole cells, classified in at least, for example, Class 435, subclass 7.2.
- XIII. Claims 73-74, drawn to a method for determining specific binding using cell extracts, classified in at least, for example, Class 435, subclass 7.1.
- XIV. Claims 75-76, drawn to a method of screening compounds to identify drugs using membrane fractions, classified in at least, for example, Class 435, subclass 7.1.
- XV. Claims 77-82, drawn to a method for identifying a compound using whole cells and activation blockade, classified in at least, for example, Class 435, subclass 7.2.
- XVI. Claims 83-84, drawn to compounds and pharmaceutical compositions, classified in at least, for example, Class 514, subclass 2.
- XVII. Claim 85, drawn to a method for detecting 5-HT<sub>4</sub> receptor on the cell surface using an antibody, classified in at least, for example, Class 435, subclass 7.2.
- XVIII. Claim 86, drawn to a method for treating an abnormal condition related to excess activity of 5-HT<sub>4</sub> receptor, classified in at least, for example, Class 514, subclass 2.
- XIX. Claim 87, drawn to a method for treating an abnormal condition alleviated by increasing the activity of 5-HT<sub>4</sub> receptor, classified in at least, for example, Class 514, subclass 2.
- XX. Claims 88-89, drawn to a method for diagnosing a predisposition to a disorder associated with expression of 5-HT<sub>4</sub> receptor alleles, classified in at least, for example, Class 435, subclass 6.

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US93/12586

XXI. Claim 90, drawn to a method of identifying a substance that alleviates abnormalities from overexpression of 5-HT<sub>4</sub> receptors using transgenic animals, classified in at least, for example, Class 424, subclass 9.

XXII. Claim 91, drawn to a method of identifying a substance that alleviates abnormalities from underexpression of 5-HT<sub>4</sub> receptors using transgenic animals, classified in at least, for example, Class 424, subclass 9.

XXIII. Claim 92, drawn to a method of treating abnormalities alleviated by reduced expression of 5-HT<sub>4</sub>, classified in at least, for example, Class 514, subclass 44.

XXIV. Claim 93, drawn to a method of treating abnormalities resulting from underexpression of 5-HT<sub>4</sub> receptor, classified in at least, for example, Class 514, subclass 2.

(

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :  C12N 1/15, 1/21, 5/10, 15/00, 15/12		A3	(11) International Publication Number: WO 94/14957  (43) International Publication Date: 7 July 1994 (07.07.94)
(21) International Application Number: PCT/US93/12586  (22) International Filing Date: 22 December 1993 (22.12.93)		(74) Agent: COBERT, Robert, J.; Cooper & Dunham, 30 Rockefeller Plaza, New York, NY 10112 (US).	
(30) Priority Data: 07/996,772 24 December 1992 (24.12.92) US		(81) Designated States: AU, CA, FL, HU, JP, KR, NO, NZ, RU, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(60) Parent Application or Grant (63) Related by Continuation US Filed on 07/996,772 (CIP) 24 December 1992 (24.12.92)		<b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(71) Applicant (for all designated States except US): SYNAPTIC PHARMACEUTICAL CORPORATION [US/US]; 215 College Road, Paramus, NJ 07652 (US).		(88) Date of publication of the international search report: 18 August 1994 (18.08.94)	
(2) Inventors; and			
(75) Inventors/Applicants (for US only): GERALD, Christophe [FR/US]; 204-B, Union Street, Ridgewood, NJ 07450 (US). HARTIG, Paul [US/US]; 19 Pheasant Run, Kinnelon, NJ 07405 (US). BRANCHEK, Theresa, A. [US/US]; 541 Martense Avenue, Teaneck, NJ 07666 (US). WEINSHANK, Richard, L. [US/US]; 302 West 87th Street, New York, NY 10024 (US).			
(54) Title: DNA ENCODING 5-HT <sub>4</sub> SEROTONIN RECEPTORS AND USES THEREOF			
(57) Abstract			
<p>This invention provides an isolated nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor and an isolated nucleic acid molecule encoding a human 5-HT<sub>4</sub> receptor, an isolated protein which is a mammalian 5-HT<sub>4</sub> receptor, an isolated protein which is a human 5-HT<sub>4</sub> receptor, vectors comprising an isolated nucleic acid molecule encoding a mammalian 5-HT<sub>4</sub> receptor, vectors comprising an isolated nucleic acid molecule encoding a human 5-HT<sub>4</sub> receptor, mammalian cells comprising such vectors, antibodies directed to the 5-HT<sub>4</sub> receptor, nucleic acid probes useful for detecting nucleic acid encoding a mammalian or human 5-HT<sub>4</sub> receptor, antisense oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a mammalian or human 5-HT<sub>4</sub> receptor, pharmaceutical compounds related to the human 5-HT<sub>4</sub> receptor, and nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian or human 5-HT<sub>4</sub> receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatments for alleviating abnormalities associated with a human 5-HT<sub>4</sub> receptor.</p>			

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GA	Gabon				

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : C12N 1/15, 1/21, 5/10, 15/00, 5/12

US C6 : 435/6, 172.3, 240.2, 252.3, 255.1, 320.1; 536/23.5, 24.3, 24.31

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 172.3, 240.2, 252.3, 255.1, 320.1; 536/23.5, 24.3, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (files 5, 155, 351, 357, 358) search terms: serotonin, receptor, DNA, 5-HT

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,985,352 (JULIUS ET AL) 15 January 1991, see Figure 1.	25-28
A, P	US, A, 5,242,822 (MARULLO ET AL) 07 September 1993, see abstract and claims.	1-37, 40-42
A	FEBS, Volume 312, Number 2,3, issued November 1992, Loric et al, "New mouse 5-HT2-like receptor", pages 203-207, see entire document.	1-37 and 40-42

Further documents are listed in the continuation of Box C.  See patent family annex.

Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other events		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

07 June 1994

Date of mailing of the international search report

JUN 20 1994

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Form PCT/ISA/210 (second sheet)(July 1992)\*

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
  
  
  
2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
  
  
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
  
  
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos. 1-37 and 40-42.

**Remark on Protest**

The additional search fees were accompanied by the applicant's protest.  
No protest accompanied the payment of additional search fees.

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as [REDACTED]:

- I. Claims 1-37 and 40-42, drawn to nucleic acid sequences for the 5-HT4 receptor, vectors, host cells, probes, antisense nucleic acids, and a method for detecting expression of a mammalian 5-HT4 receptor, classified in at least, for example, Class 536, subclass 23.5.
- II. Claims 38-39, drawn to a method of isolating a gene, classified in at least, for example, Class 435, subclass 172.3.
- III. Claims 43-44, drawn to a 5-HT4 receptor protein, classified in at least, for example, Class 530, subclass 350.
- IV. Claims 45-48, drawn to a method of production of a mammalian 5-HT4 receptor, classified in at least; for example, Class 435, subclass 69.1.
- V. Claims 49-54 and 61, drawn to an antibody to a 5-HT4 receptor protein, classified in at least, for example, Class 530, subclass 387.1.
- VI. Claim 55, drawn to a pharmaceutical composition to alleviate abnormalities resulting from overexpression of a 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.
- VII. Claim 56, drawn to a pharmaceutical composition to alleviate abnormalities resulting from underexpression of a 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.
- VIII. Claims 57-60, drawn to pharmaceutical compositions containing oligonucleotides, classified in at least, for example, Class 514, subclass 44.
- IX. Claims 62-66, drawn to transgenic animals, classified in at least, for example, Class 800, subclass 2.
- X. Claims 67-68, drawn to a method for determining physiological effects of varying levels of 5-HT4 receptors using transgenic animals, classified in at least, for example, Class 514, subclass 44.
- XI. Claims 69-70, drawn to a method for determining specific binding using whole cells, classified in at least, for example, Class 435, subclass 7.2.
- XII. Claims 71-72, drawn to a method of screening compounds to identify drugs using whole cells, classified in at least, for example, Class 435, subclass 7.2.
- XIII. Claims 73-74, drawn to a method for determining specific binding using cell extracts, classified in at least, for example, Class 435, subclass 7.1.
- XIV. Claims 75-76, drawn to a method of screening compounds to identify drugs using membrane fractions, classified in at least, for example, Class 435, subclass 7.1.
- XV. Claims 77-82, drawn to a method for identifying a compound using whole cells and activation blockade, classified in at least, for example, Class 435, subclass 7.2.
- XVI. Claims 83-84, drawn to compounds and pharmaceutical compositions, classified in at least, for example, Class 514, subclass 2.
- XVII. Claim 85, drawn to a method for detecting 5-HT4 receptor on the cell surface using an antibody, classified in at least, for example, Class 435, subclass 7.2.
- XVIII. Claim 86, drawn to a method for treating an abnormal condition related to excess activity of 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.
- XIX. Claim 87, drawn to a method for treating an abnormal condition alleviated by increasing the activity of 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.
- XX. Claims 88-89, drawn to a method for diagnosing a predisposition to a disorder associated with expression of 5-HT4 receptor alleles, classified in at least, for example, Class 435, subclass 6.

XXI. Claim 90, drawn to a method of identifying a substance that alleviates abnormalities from overexpression of 5-HT4 receptors using transgenic animals, classified in at least, for example, Class 424, subclass 9.

XXII. Claim 91, drawn to a method of identifying a substance that alleviates abnormalities from underexpression of 5-HT4 receptors using transgenic animals, classified in at least, for example, Class 424, subclass 9.

XXIII. Claim 92, drawn to a method of treating abnormalities alleviated by reduced expression of 5-HT4, classified in at least, for example, Class 514, subclass 44.

XXIV. Claim 93, drawn to a method of treating abnormalities resulting from underexpression of 5-HT4 receptor, classified in at least, for example, Class 514, subclass 2.